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(54) Title: HUMAN CALCIUM CHANNEL COMPOSITIONS AND METHODS

(57) Abstract

Isolated DNA encoding each of human calcium channel α_1-, α_2-, β- and γ-subunits, including subunits that arise as splice variants of primary transcripts, is provided. Cells and vectors containing the DNA and methods for identifying compounds that modulate the activity of human calcium channels are also provided.

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HUMAN CALCIUM CHANNEL COMPOSITIONS AND METHODS

This application is a continuation-in-part of United States Serial No. 07/868,354, filed April 10, 1992, which is a continuation-in-part of United States Serial No. 07/745,206, filed August 15, 1991, which is a continuation-in-part of United States Serial No. 07/620,250, filed November 30, 1990, which is a continuation-in-part of United States Serial No. 07/176,899, filed April 4, 1988, now abandoned, and is also a continuation-in-part of United States Serial No. 07/482,384, filed February 20, 1990, and is also a continuation-in-part of United States Serial No. 07/941,231, filed July 13, 1992, which in turn is a continuation of United States Serial No. 07/603,751, filed April 4, 1989, now abandoned.

TECHNICAL FIELD

The present invention relates to molecular biology and pharmacology. More particularly, the invention relates to calcium channel compositions and methods of making and using the same.

BACKGROUND OF THE INVENTION

Calcium channels are membrane-spanning, multi-subunit proteins that allow controlled entry of Ca^{2+} ions into cells from the extracellular fluid. Cells throughout the animal kingdom, and at least some bacterial, fungal and plant cells, possess one or more types of calcium channel.

The most common type of calcium channel is voltage dependent. "Opening" of a voltage-dependent channel to allow an influx of Ca^{2+} ions into the cells requires a depolarization to a certain level of the potential difference between the inside of the cell bearing the channel and the extracellular medium bathing the cell. The rate of influx of Ca^{2+} into the cell depends on this potential difference. All "excitable" cells in animals, such as neurons of the central nervous system (CNS), peripheral nerve cells and muscle cells, including those of skeletal muscles, cardiac muscles, and venous and arterial smooth muscles, have voltage-dependent calcium channels.

Multiple types of calcium channels have been identified in mammalian cells from various tissues, including skeletal muscle, cardiac muscle, lung, smooth muscle and brain, [see, *e.g.*, Bean, B.P.(1989) *Ann. Rev. Physiol. 51*:367-384 and Hess, P. (1990) *Ann. Rev. Neurosci. 56*:337]. The different types of calcium channels have been broadly categorized into four classes, L-, T-, N-, and P-type, distinguished by current kinetics, holding potential sensitivity and sensitivity to calcium channel agonists and antagonists.

Calcium channels are multisubunit proteins. For example, rabbit skeletal muscle calcium channel contains two large subunits, designated α_1 and α_2, which have molecular weights between about 130 and about 200 kilodaltons ("kD"), and one to three different smaller subunits of less than about 60 kD in molecular weight. At least one of the larger subunits and possibly some of the smaller subunits are glycosylated. Some of the subunits are capable of being phosphorylated. The α_1 subunit has a molecular weight of about 150 to about 170 kD when analyzed by sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) after isolation from mammalian muscle tissue and has specific binding sites for various 1,4-dihydropyridines (DHPs) and phenylalkylamines. Under non-reducing conditions (in the presence of N-ethylmaleimide), the α_2 subunit migrates in SDS-PAGE as a band corresponding to a molecular weight of about 160-190 kD. Upon reduction, a large fragment and smaller fragments are released. The β subunit of the rabbit skeletal muscle calcium channel is a phosphorylated protein that has a molecular weight of 52-65 kD as determined by SDS-PAGE analysis. This subunit is insensitive to reducing conditions. The γ subunit of the calcium channel, which is not observed in all purified preparations, appears to be a

glycoprotein with an apparent molecular weight of 30-33 kD, as determined by SDS-PAGE analysis.

In order to study calcium channel structure and function, large amounts of pure channel protein are needed. Because of the complex nature of these multisubunit proteins, the varying concentrations of calcium channels in tissue sources of the protein, the presence of mixed populations of calcium channels in tissues, difficulties in obtaining tissues of interest, and the modifications of the native protein that can occur during the isolation procedure, it is extremely difficult to obtain large amounts of highly purified, completely intact calcium channel protein.

Characterization of a particular type of calcium channel by analysis of whole cells is severely restricted by the presence of mixed populations of different types of calcium channels in the majority of cells. Single-channel recording methods that are used to examine individual calcium channels do not reveal any information regarding the molecular structure or biochemical composition of the channel. Furthermore, in performing this type of analysis, the channel is isolated from other cellular constituents that might be important for natural functions and pharmacological interactions.

Characterization of the gene or genes encoding calcium channels provides another means of characterization of different types of calcium channels. The amino acid sequence determined from a complete nucleotide sequence of the coding region of a gene encoding a calcium channel protein represents the primary structure of the protein. Furthermore, secondary structure of the calcium channel protein and the relationship of the protein to the membrane may be predicted based on analysis of the primary structure. For instance, hydropathy plots of the α_1 subunit protein of the rabbit skeletal muscle calcium channel

indicate that it contains four internal repeats, each containing six putative transmembrane regions [Tanabe, T. *et al.* (1987) *Nature 328*:313].

The cDNA and corresponding amino acid sequences of the α_1, α_2, β and γ subunits of the rabbit skeletal muscle calcium channel [see, Tanabe *et al.* (1987) *Nature 328*:313-318; International Application No. WO 89/09834, which is U.S. Application Serial No. 07/603,751, which is a continuation-in-part of U.S. Application Serial No. 07/176,899; Ruth *et al.* (1989) *Science 245*:1115-1118; and U.S. Patent Application Serial No. 482,384, filed February 20, 1990] have been determined. The cDNA and corresponding amino acid sequences of α_1 subunits of rabbit cardiac muscle [Mikami, A. *et al.* (1989) *Nature 340*:230-233] and lung [Biel, M. (1990) *FEBS Letters 269*:409-412] calcium channels have been determined.

In addition, a cDNA clone encoding a rabbit brain calcium channel (designated the BI channel) has been isolated [Mori, Y. *et al.* (1991) *Nature 350*:398-402]. Partial cDNA clones encoding portions of several different subtypes, referred to as rat brain class A, B, C and D, of the calcium channel α_1 subunit have been isolated from rat brain cDNA libraries [Snutch, T. *et al.* (1990) *Proc. Natl. Acad. Sci. USA 87*:3391-3395]. More recently full-length rat brain class A [Starr, T. *et al.* (1991) *Proc. Natl. Acad. Sci. USA 88*:5621-5625] and class C [Snutch, T. *et al.* (1991) *Neuron 7*:45-57] cDNA clones have been isolated. Although the amino acid sequence encoded by the rat brain class C DNA is approximately 95% identical to that encoded by the rabbit cardiac muscle calcium channel α_1 subunit-encoding DNA, the amino acid sequence encoded by the rat brain class A DNA shares only 33% sequence identity with the amino acid sequence encoded by the rabbit skeletal or cardiac muscle α_1 subunit-encoding DNA. A cDNA clone encoding another rat brain calcium channel α_1 subunit has

also been obtained [Hui, A. *et al.* (1991) *Neuron* 7:35-44]. The amino acid sequence encoded by this clone is ~70% homologous to the proteins encoded by the rabbit skeletal and cardiac muscle calcium channel DNA. A cDNA clone closely related to the rat brain class C α_1 subunit-encoding cDNA and sequences of partial cDNA clones closely related to other partial cDNA clones encoding apparently different calcium channel α_1 subunits have also been isolated [see Snutch, T. *et al.* (1991) *Neuron* 7:45-57; Perez-Reyes, E. *et al.* (1990) *J. Biol. Chem.* 265:20430; and Hui, A. *et al.* (1991) *Neuron* 7:35-44]. DNA clones encoding other calcium channels have also been identified and isolated.

Expression of cDNA encoding calcium channel subunits has been achieved with several of the different rabbit or rat α_1 subunit cDNA clones discussed above. Voltage-dependent calcium currents have been detected in murine L cells transfected with DNA encoding the rabbit skeletal muscle calcium channel α_1 subunit [Perez-Reyes *et al.* (1989) *Nature* 340:233-236 (1989)]. These currents were enhanced in the presence of the calcium channel agonist Bay K 8644. Bay K 8644-sensitive Ba^{2+} currents have been detected in oöcytes injected with *in vitro* transcripts of the rabbit cardiac muscle calcium channel α_1 subunit cDNA [Mikami, A. *et al.* (1989) *Nature* 340:230-233]. These currents were substantially reduced in the presence of the calcium channel antagonist nifedipine. Barium currents of an oöcyte co-injected with RNA encoding the rabbit cardiac muscle calcium channel α_1 subunit and the RNA encoding the rabbit skeletal muscle calcium channel α_2 subunit were more than 2-fold larger than those of oöcytes injected with transcripts of the rabbit cardiac calcium channel α_1 subunit-encoding cDNA. Similar results were obtained when oöcytes were co-injected with RNA encoding the rabbit lung calcium channel α_1 subunit and the rabbit skeletal muscle calcium channel α_2 subunit. The barium current was greater

than that detected in oöcytes injected only with RNA encoding the rabbit lung calcium channel α_1 subunit [Biel, M. *et al.* (1990) *FEBS Letters 269*:409-412]. Inward barium currents have been detected in oöcytes injected with *in vitro* RNA transcripts encoding the rabbit brain BI channel [Mori *et al.* (1991) *Nature 350*:398-402]. These currents were increased by two orders of magnitude when *in vitro* transcripts of the rabbit skeletal muscle calcium channel α_2, β, or α_2, β and γ subunits were co-injected with transcripts of the BI-encoding cDNA. Barium currents in oöcytes co-injected with transcripts encoding the BI channel and the rabbit skeletal muscle calcium channel α_2 and β were unaffected by the calcium channel antagonists nifedipine or ω-CgTx and inhibited by Bay K 8644 and crude venom from *Agelenopsis aperta*.

The results of studies of recombinant expression of rabbit calcium channel α_1 subunit-encoding cDNA clones and transcripts of the cDNA clones indicate that the α_1 subunit forms the pore through which calcium enters cells. The relevance of the barium currents generated in these recombinant cells to the actual current generated by calcium channels containing as one component the respective α_1 subunits *in vivo* is unclear. In order to completely and accurately characterize and evaluate different calcium channel types, however, it is essential to examine the functional properties of recombinant channels containing all of the subunits as found *in vivo*. Although there has been limited success in expressing DNA encoding rabbit and rat calcium channel subunits, far less has been achieved with respect to human calcium channels. Little is known about human calcium channel structure and function and gene expression. An understanding of the structure and function of human calcium channels would permit identification of substances that, in some manner, modulate the activity of

calcium channels and that have potential for use in treating such disorders.

Because calcium channels are present in various tissues and have a central role in regulating intracellular calcium ion concentrations, they are implicated in a number of vital processes in animals, including neurotransmitter release, muscle contraction, pacemaker activity, and secretion of hormones and other substances. These processes appear to be involved in numerous human disorders, such as CNS and cardiovascular diseases. Calcium channels, thus, are also implicated in numerous disorders. A number of compounds useful for treating various cardiovascular diseases in animals, including humans, are thought to exert their beneficial effects by modulating functions of voltage-dependent calcium channels present in cardiac and/or vascular smooth muscle. Many of these compounds bind to calcium channels and block, or reduce the rate of, influx of Ca^{2+} into the cells in response to depolarization of the cell membrane.

An understanding of the pharmacology of compounds that interact with calcium channels in other organ systems, such as the CNS, may aid in the rational design of compounds that specifically interact with subtypes of human calcium channels to have desired therapeutic effects, such as in the treatment of neurodegenerative and cardiovascular disorders. Such understanding and the ability to rationally design therapeutically effective compounds, however, have been hampered by an inability to independently determine the types of human calcium channels and the molecular nature of individual subtypes, particularly in the CNS, and by the unavailability of pure preparations of specific channel subtypes to use for evaluation of the specificity of calcium channel-effecting compounds. Thus, identification of DNA encoding human calcium channel subunits and the use of such DNA for

expression of calcium channel subunits and functional calcium channels would aid in screening and designing therapeutically effective compounds.

Therefore, it is an object herein, to provide DNA encoding specific calcium channel subunits and to provide eukaryotic cells bearing recombinant tissue-specific or subtype- specific calcium channels. It is also an object to provide assays for identification of potentially therapeutic compounds that act as calcium channel antagonists and agonists.

SUMMARY OF THE INVENTION

Eukaryotic cells containing heterologous DNA encoding one or more calcium channel subunits, particularly human calcium channel subunits, or containing RNA transcripts of DNA clones encoding one or more of the subunits are provided. In preferred embodiments, the cells contain DNA or RNA encoding a human α_1 subunit, preferably at least an α_{1D} or α_{1B} subunit. In more preferred embodiments, the cells contain DNA or RNA encoding additional heterologous subunits, including at least one β, α_2 or γ subunits are included. In such embodiments, eukaryotic cells stably or transiently transfected with any combination of one, two, three or four of the subunit-encoding cDNA clones, such as α_1, $\alpha_1 + \beta$, $\alpha_1 + \beta + \alpha_2$, are provided. In more preferred embodiments, the subunits encoded by the heterologous DNA are human subunits.

In preferred embodiments, the cells express such heterologous calcium channel subunits and include one or more of the subunits in membrane spanning heterologous calcium channels. In more preferred embodiments, the eukaryotic cells express functional, heterologous calcium channels that are capable of gating the passage of calcium channel selective ions and/or binding compounds that, at physiological concentrations, modulate the activity of the heterologous calcium channel. In certain embodiments, the

heterologous calcium channels include at least one heterologous calcium channel subunit. In most preferred embodiments, the calcium channels that are expressed on the surface of the eukaryotic cells are composed substantially or entirely of subunits encoded by the heterologous DNA or RNA. In preferred embodiments, the heterologous calcium channels of such cells are distinguishable from any endogenous calcium channels of the host cell.

In certain embodiments the recombinant eukaryotic cells that contain the heterologous DNA encoding the calcium channel subunits are produced by transfection with DNA encoding one or more of the subunits or are injected with RNA transcripts of cDNA encoding one or more of the calcium channel subunits. The DNA may be introduced as a linear DNA fragment or may be included in an expression vector for stable or transient expression of the subunit-encoding DNA. Vectors containing DNA encoding human calcium channel subunits are also provided.

The eukaryotic cells that express heterologous calcium channels may be used in assays for calcium channel function or, in the case of cells transformed with fewer subunit-encoding nucleic acids than necessary to constitute a functional recombinant human calcium channel, such cells may be used to assess the effects of additional subunits on calcium channel activity. The additional subunits can be provided by subsequently transfecting such a cell with one or more DNA clones or RNA transcripts encoding human calcium channel subunits.

The recombinant eukaryotic cells that express membrane spanning heterologous calcium channels may be used in methods for identifying compounds that modulate calcium channel activity. In particular, the cells are used in assays that identify agonists and antagonists of calcium channel activity in humans and/or assessing the

contribution of the various calcium channel subunits to the transport and regulation of transport of calcium ions.

Assays using the eukaryotic cells for identifying compounds that modulate calcium channel activity are provided.

Isolated and purified DNA fragments that encode human calcium channel subunits are provided. DNA encoding α_1 subunits of a human calcium channel, and RNA, encoding such subunits, made upon transcription of such DNA are provided. In particular, DNA fragments encoding α_1 subunits of voltage-dependent human calcium channels (VDCCs) type A, type B (also referred to as VDCC IV), type C (also referred to as VDCC II) and type D (also referred to as VDCC III) are provided.

In particular, DNA encoding an α_{1D} subunit that includes the amino acids substantially as set forth as residues 10-2161 of sequence ID No. 1 is provided. DNA encoding an α_{1D} subunit includes substantially the amino acids set forth as amino acids 1-34 in sequence ID No. 2 in place of amino acids 373-406 of SEQ ID No. 1 is also provided. DNA encoding an α_{1C} subunit that includes the amino acids substantially as set forth in sequence ID No. 3 or sequence ID No. 6 and DNA encoding an α_{1B} subunit that includes an amino acid sequence substantially as set forth in sequence ID No. 7 or in sequence ID No. 8 is also provided. A phage lysate of an *E. coli* host containing DNA encoding α_{1A} have been deposited in the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under Accession No. in accord with the Budapest Treaty. The DNA in such phage includes a DNA fragment having the sequence set forth in SEQ ID No. 21. This fragment hybridizes to DNA encoding α_{1A} but not to DNA encoding α_{1B}.

DNA encoding α_2 subunits of a human calcium channel, and RNA encoding such subunits, made upon transcription of

such a DNA are provided. DNA encoding splice variants of the α_2 subunit, including tissue specific splice variants, are also provided. In particular, DNA encoding the α_{2a}-α_{2c} subunit subtypes is provided. In particularly preferred embodiments, the DNA encoding the α_2 subunit is produced by alternative processing of a primary transcript that includes DNA encoding the amino acids set forth in SEQ ID 11 and the DNA of SEQ ID No. 13 inserted between nucleotides 1624 and 1625 of SEQ ID No. 11.

Isolated and purified DNA fragments encoding human calcium channel β subunits, including DNA encoding β_1 subunit splice variants and the β_3 subunit and is provided. In particular, DNA encoding the β_1 and β_3 subunits, including the β_1 subunit splice variants β_{1-1}-β_{1-5}, is provided. RNA, encoding β subunits, made upon transcription of the DNA is also provided. *Escherichia coli* (*E. coli*) containing plasmids containing DNA encoding β_3 have been deposited in accord with the Budapest Treaty under Accession No. 69048 at the American Type Culture Collection. A partial sequence of the deposited clone is set forth in SEQ ID No. 19 (sequence from the 5′ end) and SEQ ID No. 20 (sequence from the 3′ end).

DNA encoding β subunits that are produced by alternative processing of a primary transcript encoding a β subunit, including a transcript that includes DNA encoding the amino acids set forth in SEQ ID No. 9 or including a primary transcript that encodes β_3 as deposited under ATCC Accession No. 69048, but lacking and including alternative exons are provided or may be constructed from the DNA provided herein. For example, DNA encoding a β subunit that is produced by alternative processing of a primary transcript that includes DNA encoding the amino acids set forth in SEQ ID No. 9, but including the DNA set forth in SEQ ID No. 12 inserted in place of nucleotides 615-781 of SEQ ID No. 9 is also provided. DNA encoding β

subunits that are encoded by transcripts that have the sequence set forth in SEQ ID No. 9 including the DNA set forth in SEQ ID No. 12 inserted in place of nucleotides 615-781 of SEQ ID No. 9, but that lack one or more of the following sequences of nucleotides: nucleotides 14-34 of SEQ ID No. 12, nucleotides 13-34 of SEQ ID No. 12, nucleotides 35-55 of SEQ ID No 12, nucleotides 56-190 of SEQ ID No. 12 and nucleotides 191-271 of SEQ ID No. 12 are also provided.

DNA encoding γ subunits of human calcium channels is also provided. RNA, encoding γ subunits, made upon transcription of the DNA are also provided. In particular, DNA containing the sequence of nucleotides set forth in SEQ ID No. 14 is provided.

Full-length DNA clones and corresponding RNA transcripts, encoding the α_1, including α_{1D}, α_{1B}, α_2 and β subunits, including β_{1-1}-β_{1-5}, of human calcium channels are provided. Also provided are DNA clones encoding substantial portions of the α_{1A}, α_{1C}, β_3 and γ subunits of voltage-dependent human calcium channels for the preparation of full-length DNA clones encoding the full-length α_{1A}, α_{1C}, β_3 and γ subunits.

Nucleic acid probes containing at least about 14 contiguous nucleotides of α_{1D}, α_{1C}, α_{1B}, α_{1A}, α_2, β, including β_1 splice variants and β_3, and γ subunit-encoding DNA are provided. Methods using the probes for the isolation and cloning of calcium channel subunit-encoding cDNA, including splice variants within tissues and inter-tissue variants are also provided.

Purified human calcium channel subunits and purified human calcium channels are provided. The subunits and channels can be isolated from a eukaryotic cell transfected with DNA that encodes the subunit.

In another embodiment, immunoglobulins or antibodies obtained from the serum of an animal immunized with a

substantially pure preparation of a human calcium channel, human calcium channel subunit or epitope-containing fragment of a human calcium subunit are provided. Monoclonal antibodies produced using a human calcium channel, human calcium channel subunit or epitope-containing fragment thereof as an immunogen are also provided. *E. coli* fusion proteins including a fragment of a human calcium channel subunit may also be used as immunogen. Such fusion proteins may contain a bacterial protein or portion thereof, such as the *E. coli* TrpE protein, fused to a calcium channel subunit peptide. The immunoglobulins that are produced using the calcium channel subunits or purified calcium channels as immunogens have, among other properties, the ability to specifically and preferentially bind to and/or cause the immunoprecipitation of a human calcium channel or a subunit thereof which may be present in a biological sample or a solution derived from such a biological sample.

A diagnostic method for determining the presence of Lambert Eaton Syndrome (LES) in a human based on immunological reactivity of LES immunoglobulin G (IgG) with a human calcium channel subunit or a eukaryotic cell which expresses a recombinant human calcium channel or a subunit thereof is also provided. In particular, an immunoassay method for diagnosing Lambert-Eaton Syndrome in a person by combining serum or an IgG fraction from the person (test serum) with calcium channel proteins, including the α and β subunits, and ascertaining whether antibodies in the test serum react with one or more of the subunits, or a recombinant cell which expresses one or more of the subunits to a greater extent than antibodies in control serum, obtained from a person or group of persons known to be free of the Syndrome, is provided. Any immunoassay procedure known in the art for detecting antibodies against a given antigen in serum can be employed in the method.

DETAILED DESCRIPTION OF THE INVENTION

Definitions:

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents and publications referred to herein are incorporated by reference herein.

Reference to each of the calcium channel subunits includes the subunits that are specifically disclosed herein and human calcium channel subunits encoded by DNA that can be isolated by using the DNA disclosed as probes and screening an appropriate human cDNA or genomic library under at least low stringency. Such DNA also includes DNA that encodes proteins that have about 40% homology to any of the subunits proteins described herein or DNA that hybridizes under conditions of at least low stringency to the DNA provided herein and the protein encoded by such DNA exhibits additional identifying characteristics, such as function or molecular weight.

It is understood that subunits that are encoded by transcripts that represent splice variants of the disclosed subunits or other such subunits may exhibit less than 40% overall homology to any single subunit, but will include regions of such homology to one or more such subunits. It is also understood that 40% homology refers to proteins that share approximately 40% of their amino acids in common or that share somewhat less, but include conservative amino acid substitutions, whereby the activity of the protein is not substantially altered.

As used herein, the α_1 subunits types, encoded by different genes, are designated as type α_{1A}, α_{1B}, α_{1C}, and α_{1D}. These types may also be also referred to as VDCC IV for α_{1B}, VDCC II for α_{1C} and VDCC III for α_{1D}. Subunit subtypes, which are splice variants, are referred to, for example as α_{1B-1}, α_{1B-2}, α_{1C-1} etc.

Thus, as used herein, DNA encoding the α_1 subunit refers to DNA that hybridizes to the DNA provided herein under conditions of at least low stringency or encodes a subunit that has roughly about 40% homology to protein encoded by DNA disclosed herein that encodes an α_1 subunit of a human calcium. An α_1 subunit may be identified by its ability to form a calcium channel. Typically, α_1 subunits have molecular weights greater than at least about 120 kD. The activity of a calcium channel may be assessed *in vitro* by methods known to those of skill in the art, including the electrophysiological and other methods described herein. Typically, α_1 subunits include regions to which one or more modulators of calcium channel activity, such as a 1,4 DHP or ω-CgTx, interact directly or indirectly. Types of α_1 subunits may be distinguished by any method known to those of skill in the art, including on the basis of binding specificity. For example, it has been found herein that α_{1B} subunits participate in the formation N-type channels, α_{1D} subunits participate in the formation of L-type channels, and α_{1A} subunits appear to participate in the formation of channels that exhibit characteristics typical of P-type channels. Thus, for example, the activity of channels that contain the α_{1B} subunit are insensitive to 1,4 DHPs; whereas the activity of channels that contain the α_{1D} subunit are modulated or altered by a 1,4 DHP. Types and subtypes of α_1 subunits may be characterized on the basis of the effects of such modulators on the subunit or a channel containing the subunit as well as differences in currents and current kinetics produced by calcium channels containing the subunit.

As used herein, an α_2 subunit is encoded by DNA that hybridizes to the DNA provided herein under conditions of low stringency or encodes a protein that has about 40% homology with that disclosed herein. Such DNA encodes a protein that typically has a molecular weight greater than

about 120 kD, but does not form a calcium channel in the absence of an α_1 subunit, and may alter the activity of a calcium channel that contains an α_1 subunit. Subtypes of the α_2 subunit that arise as splice variants are designated by lower case letter, such as α_{2a}, . . . α_{2c}. In addition, the α_2 subunit and the large fragment produced under reducing conditions appear to be glycosylated with at least N-linked sugars and do not specifically bind to the 1,4-DHPs and phenylalkylamines that specifically bind to the α_1 subunit. The smaller fragment, the C-terminal fragment, is referred to as the δ subunit and includes amino acids from about 946 (SEQ ID No. 11) through about the C-terminus. This fragment may dissociate from the remaining portion of α_2 when the α_2 subunit is exposed to reducing conditions.

As used herein, a β subunit is encoded by DNA that hybridizes to the DNA provided herein under conditions of low stringency or encodes a protein that has about 40% homology with that disclosed herein and is a protein that typically has a molecular weight lower than the α subunits and on the order of about 50-80 kD, does not form a detectable calcium channel in the absence of an α_1 subunit, but may alter the activity of a calcium channel that contains an α_1 subunit or that contains an α_1 and α_2 subunit.

Types of the β subunit that are encoded by different genes are designated with subscripts, such as β_1 and β_3. Subtypes of β subunits that arise as splice variants of a particular type are designated with a numerical subscript referring to the subtype and to the variant. Such subtypes include, but are not limited to the β_1 splice variants, including β_{1-1}-β_{1-5}.

As used herein, a γ subunit is a subunit encoded by DNA disclosed herein as encoding the γ subunit and may be isolated and identified using the DNA disclosed herein as a probe by hybridization or other such method known to

those of skill in the art, whereby full-length clones encoding a γ subunit may be isolated or constructed. A γ subunit will be encoded by DNA that hybridizes to the DNA provided herein under conditions of low stringency or exhibits sufficient sequence homology to encode a protein that has about 40% homology with the γ subunit described herein.

Thus, one of skill in the art, in light of the disclosure herein, can identify DNA encoding α_1, α_2, β, δ and calcium channel subunits, including types encoded by different genes and subtypes that represent splice variants. For example, DNA probes based on the DNA disclosed herein may be used to screen an appropriate library, including a genomic or cDNA library, and obtain DNA in one or more clones that includes an open reading fragment that encodes an entire protein. Subsequent to screening an appropriate library with the DNA disclosed herein, the isolated DNA can be examined for the presence of an open reading frame from which the sequence of the encoded protein may be deduced. Determination of the molecular weight and comparison with the sequences herein should reveal the identity of the subunit as an α_1, α_2 etc. subunit. Functional assays may, if necessary, be used to determine whether the subunit is an α_1, α_2 subunit or β subunit.

For example, DNA encoding α_{1A} may be isolated by screening an appropriate library with DNA, encoding all or a portion of the human α_{1A} subunit, isolated from the phage deposited under ATCC Accession No. , including screening with an oligonucleotide having the sequence set forth in SEQ ID No. 21. Similarly, DNA encoding β_3 may be isolated by screening a human cDNA library with DNA probes prepared from the plasmid β1.42 deposited under ATCC Accession No. 69048 or probes having sequences prepared according to the sequences set forth in SEQ ID Nos. 19 and

20. Any method known to those of skill in the art for isolation and identification of DNA and preparation of full-length genomic or cDNA clones, including methods exemplified herein, may be used.

The subunit encoded by isolated DNA may be identified by comparison with the DNA and amino acid sequences of the subunits provided herein. Splice variants share extensive regions of homology, but include non-homologous regions, subunits encoded by different genes share a uniform distribution of non-homologous sequences.

As used herein, a splice variant refers to a variant produced by differential processing of a primary transcript of genomic DNA that results in more than one type of mRNA. Splice variants may occur within a single tissue type or among tissues (tissue-specific variants). Thus, cDNA clones that encode calcium channel subunit subtypes that have regions of identical amino acids and regions of different amino acid sequences are referred to herein as "splice variants".

As used herein, a "calcium channel selective ion" is an ion that is capable of flowing through, or being blocked from flowing through, a calcium channel which spans a cellular membrane under conditions which would substantially similarly permit or block the flow of Ca^{2+}. Ba^{2+} is an example of an ion which is a calcium channel selective ion.

As used herein, a compound that modulates calcium channel activity is one that affects the ability of the calcium channel to pass calcium channel selective ions or affects other detectable calcium channel features, such as current kinetics. Such compounds include calcium channel antagonists and agonists and compounds that exert their effect on the activity of the calcium channel directly or indirectly.

As used herein, a "substantially pure" subunit or protein is a subunit or protein that is sufficiently free of other polypeptide contaminants to appear homogeneous by SDS-PAGE or to be unambiguously sequenced.

As used herein, heterologous or foreign DNA and RNA are used interchangeably and refer to DNA or RNA that does not occur naturally as part of the genome in which it is present or which is found in a location or locations in the genome that differ from that in which it occurs in nature. It is DNA or RNA that is not endogenous to the cell and has been artificially introduced into the cell. Examples of heterologous DNA include, but are not limited to, DNA that encodes a calcium channel subunit and DNA that encodes RNA or proteins that mediate or alter expression of endogenous DNA by affecting transcription, translation, or other regulatable biochemical processes. The cell that expresses the heterologous DNA, such as DNA encoding the calcium channel subunit, may contain DNA encoding the same or different calcium channel subunits. The heterologous DNA need not be expressed and may be introduced in a manner such that it is integrated into the host cell genome or is maintained episomally.

As used herein, operative linkage of heterologous DNA to regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences, refers to the functional relationship between such DNA and such sequences of nucleotides. For example, operative linkage of heterologous DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA in reading frame.

As used herein, isolated, substantially pure DNA refers to DNA fragments purified according to standard techniques employed by those skilled in the art [see, e.g., Maniatis *et al.* (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY].

As used herein, expression refers to the process by which nucleic acid is transcribed into mRNA and translated into peptides, polypeptides, or proteins. If the nucleic acid is derived from genomic DNA, expression may, if an appropriate eukaryotic host cell or organism is selected, include splicing of the mRNA.

As used herein, vector or plasmid refers to discrete elements that are used to introduce heterologous DNA into cells for either expression of the heterologous DNA or for replication of the cloned heterologous DNA. Selection and use of such vectors and plasmids are well within the level of skill of the art.

As used herein, expression vector includes vectors capable of expressing DNA fragments that are in operative linkage with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or may integrate into the host cell genome.

As used herein, a promoter region refers to the portion of DNA of a gene that controls transcription of DNA to which it is operatively linked. The promoter region includes specific sequences of DNA that are sufficient for

RNA polymerase recognition, binding and transcription initiation. This portion of the promoter region is referred to as the promoter. In addition, the promoter region includes sequences that modulate this recognition, binding and transcription initiation activity of the RNA polymerase. These sequences may be *cis* acting or may be responsive to *trans* acting factors. Promoters, depending upon the nature of the regulation, may be constitutive or regulated.

As used herein, a recombinant eukaryotic cell is a eukaryotic cell that contains heterologous DNA or RNA.

As used herein, a recombinant or heterologous calcium channel refers to a calcium channel that contains one or more subunits that are encoded by heterologous DNA that has been introduced into and expressed in a eukaryotic cells that expresses the recombinant calcium channel. A recombinant calcium channel may also include subunits that are produced by DNA endogenous to the cell. In certain embodiments, the recombinant or heterologous calcium channel may contain only subunits that are encoded by heterologous DNA.

As used herein, "functional" with respect to a recombinant or heterologous calcium channel means that the channel is able to provide for and regulate entry of calcium channel selective ions, including, but not limited to, Ca^{2+} or Ba^{2+}, in response to a stimulus and/or bind ligands with affinity for the channel. Preferably such calcium channel activity is distinguishable, such as electrophysiological, pharmacological and other means known to those of skill in the art, from any endogenous calcium channel activity that in the host cell.

As used herein, a peptide having an amino acid sequence substantially as set forth in a particular SEQ ID No. includes peptides that have the same function but may include minor variations in sequence, such as conservative

amino acid changes or minor deletions or insertions that do not alter the activity of the peptide. The activity of a calcium channel receptor subunit peptide refers to its ability to form functional calcium channels with other such subunits.

As used herein, a physiological concentration of a compound is that which is necessary and sufficient for a biological process to occur. For example, a physiological concentration of a calcium channel selective ion is a concentration of the calcium channel selective ion necessary and sufficient to provide an inward current when the channels open.

As used herein, activity of a calcium channel refers to the movement of a calcium selective ion through a calcium channel. Such activity may be measured by any method known to those of skill in the art, including, but not limited to, measurement of the amount of current which flows through the recombinant channel in response to a stimulus.

As used herein, a "functional assay" refers to an assay that identifies functional calcium channels. A functional assay, thus, is an assay to assess function.

As understood by those skilled in the art, assay methods for identifying compounds, such as antagonists and agonists, that modulate calcium channel activity, generally requires comparison to a control. One type of a "control" cell or "control" culture is a cell or culture that is treated substantially the same as the cell or culture exposed to the test compound except that the control culture is not exposed to the test compound. Another type of a "control" cell or "control" culture may be a cell or a culture of cells which are identical to the transfected cells except the cells employed for the control culture do not express functional calcium channels. In this situation, the response of test cell to the test compound

compared to the response (or lack of response) of the receptor-negative cell to the test compound, when cells or cultures of each type of cell are exposed to substantially the same reaction conditions in the presence of the compound being assayed. For example, in methods that use patch clamp electrophysiological procedures, the same cell can be tested in the presence and absence of the test compound, by changing the external solution bathing the cell as known in the art.

Assays

Assays for identifying compounds that modulate calcium channel activity

In vitro methods for identifying compounds, such as calcium channel agonist and antagonists, that modulate the activity of calcium channels using eukaryotic cells that express heterologous human calcium channels are provided.

In particular, the assays use eukaryotic cells that express heterologous human calcium channel subunits encoded by heterologous DNA provided herein, for screening potential calcium channel agonists and antagonists which are specific for human calcium channels and particularly for screening for compounds that are specific for particular human calcium channel subtypes. Such assays may be used in conjunction with methods of rational drug design to select among agonists and antagonists, which differ slightly in structure, those particularly useful for modulating the activity of human calcium channels, and to design or select compounds that exhibit subtype- or tissue-specific calcium channel antagonist and agonist activities.

These assays should accurately predict the relative therapeutic efficacy of a compound for the treatment of certain disorders in humans. In addition, since subtype- and tissue-specific calcium channel subunits are provided, cells with tissue- specific or subtype-specific recombinant calcium channels may be prepared and used in assays for

identification of human calcium channel tissue- or subtype-specific drugs.

The assays involve contacting the cell membrane of a recombinant eukaryotic cell which expresses at least one subunit of a human calcium channel, preferably at least an α_1 subunit of a human calcium channel, with a test compound and measuring the ability of the test compound to specifically bind to the membrane or alter or modulate the activity of a heterologous calcium channel on the membrane.

In preferred embodiments, the assay uses a recombinant cell which has a calcium channel containing an α_1 subunit of a human calcium channel in combination with a β-subunit of a human calcium channel and/or an α_2 subunit of a human calcium channel. Recombinant cells expressing heterologous calcium channels containing each of the α_1, β and α_2 human subunits, and, optionally, a γ subunit of a human calcium channel are especially preferred for use in such assays.

In certain embodiments, the assays for identifying compounds that modulate calcium channel activity are practiced by measuring the calcium channel activity of a eukaryotic cell having a heterologous, functional calcium channel when such cell is exposed to a solution containing the test compound and a calcium channel selective ion and comparing the measured calcium channel activity to the calcium channel activity of the same cell or a substantially identical control cell in a solution not containing the test compound. The cell is maintained in a solution having a concentration of calcium channel selective ions sufficient to provide an inward current when the channels open. Especially preferred for use, is a recombinant cell expressing calcium channels that include each of the α_1, β and α_2 human subunits, and, optionally, a γ subunit of a human calcium channel. Methods for practicing such assays are known to those of skill in the art. For example, for similar methods applied with *Xenopus*

laevis oöcytes and acetylcholine receptors, see, Mishina *et al.* [(1985) *Nature 313*:364] and, with such oöcytes and sodium channels [see, Noda *et al.* (1986) *Nature 322*:826-828]. For similar studies which have been carried out with the acetylcholine receptor, see, *e.g.*, Claudio *et al.* [(1987) *Science 238*:1688-1694].

The assays thus use cells, provided herein, that express heterologous functional calcium channels and measure functionally, such as electrophysiologically, the ability of a test compound to potentiate, antagonize or otherwise modulate the magnitude and duration of the flow of calcium channel selective ions, such as Ca^{++} or Ba^{++}, through the heterologous functional channel. The amount of current which flows through the recombinant calcium channels of a cell may be determined directly, such as electrophysiologically, or by monitoring an independent reaction which occurs intracellularly and which is directly influenced in a calcium (or other) ion dependent manner.

Any method for assessing the activity of a calcium channel may be used in conjunction with the cells and assays provided herein. For example, in one embodiment of the method for testing a compound for its ability to modulate calcium channel activity, the amount of current is measured by its modulation of a reaction which is sensitive to calcium channel selective ions and uses a eukaryotic cell which expresses a heterologous calcium channel and also contains a transcriptional control element operatively linked for expression to a structural gene that encodes an indicator protein. The transcriptional control element used for transcription of the indicator gene is responsive in the cell to a calcium channel selective ion, such as Ca^{2+} and Ba^{+}. The details of such transcriptional based assays are described in commonly owned PCT International Patent Application No. PCT/US91/5625, filed August 7, 1991, which claims priority to copending commonly owned U.S.

Application Serial No. 07/ 563,751, filed August 7, 1990, the contents of which applications are herein incorporated by reference thereto.

Assays for diagnosis of LES

LES is an autoimmune disease characterized by an insufficient release of acetylcholine from motor nerve terminals which normally are responsive to nerve impulses. Immunoglobulins (IgG) from LES patients block individual voltage-dependent calcium channels and thus inhibit calcium channel activity [Kim and Neher, *Science 239*:405-408 (1988)]. A diagnostic assay for Lambert Eaton Syndrome (LES) is provided herein. The diagnostic assay for LES relies on the immunological reactivity of LES IgG with the human calcium channels or particular subunits alone or in combination or expressed on the surface of recombinant cells. For example, such an assay may be based on immunoprecipitation of LES IgG by the human calcium channel subunits and cells that express such subunits provided herein.

Identification and isolation of DNA encoding human calcium channel subunits

Methods for identifying and isolating DNA encoding α_1, α_2, β and γ subunits of human calcium channels are provided.

Identification and isolation of such DNA may be accomplished by hybridizing, under appropriate conditions, at least low stringency whereby DNA that encodes the desired subunit is isolated, restriction enzyme-digested human DNA with a labeled probe having at least 14 nucleotides and derived from any contiguous portion of DNA having a sequence of nucleotides set forth herein by sequence identification number. Once a hybridizing fragment is identified in the hybridization reaction, it can be cloned employing standard cloning techniques known to those of skill in the art. Full-length clones may be identified by the presence of a complete open reading frame and the identity of the encoded protein verified by

sequence comparison with the subunits provided herein and by functional assays to assess calcium channel forming ability or other function. This method can be used to identify genomic DNA encoding the subunit or cDNA encoding splice variants of human calcium channel subunits generated by alternative splicing of the primary transcript of genomic subunit DNA. For instance, DNA, cDNA or genomic DNA, encoding a calcium channel subunit may be identified by hybridization to a DNA probe and characterized by methods known to those of skill in the art, such as restriction mapping and DNA sequencing, and compared to the DNA provided herein in order to identify heterogeneity or divergence in the sequences the DNA. Such sequence differences may indicate that the transcripts from which the cDNA was produced result from alternative splicing of a primary transcript, if the non-homologous and homologous regions are clustered, or from a different gene if the non-homologous regions are distributed throughout the cloned DNA.

Any suitable method for isolating genes using the DNA provided herein may be used. For example, oligonucleotides corresponding to regions of sequence differences have been used to isolate, by hybridization, DNA encoding the full-length splice variant and can be used to isolate genomic clones. A probe, based on a nucleotide sequence disclosed herein, which encodes at least a portion of a subunit of a human calcium channel, such as a tissue-specific exon, may be used as a probe to clone related DNA, to clone a full-length cDNA clone or genomic clone encoding the human calcium channel subunit.

Labeled, including, but not limited to, radioactively or enzymatically labeled, RNA or single-stranded DNA of at least 14 substantially contiguous bases, preferably at least 30 contiguous bases of a nucleic acid which encodes at least a portion of a human calcium channel subunit, the

sequence of which nucleic acid corresponds to a segment of a nucleic acid sequence disclosed herein by reference to a SEQ ID No. are provided. Such nucleic acid segments may be used as probes in the methods provided herein for cloning DNA encoding calcium channel subunits. See, generally, Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press.

In addition, nucleic acid amplification techniques, which are well known in the art, can be used to locate splice variants of calcium channel subunits by employing oligonucleotides based on DNA sequences surrounding the divergent sequence primers for amplifying human RNA or genomic DNA. Size and sequence determinations of the amplification products can reveal splice variants. Furthermore, isolation of human genomic DNA sequences by hybridization can yield DNA containing multiple exons, separated by introns, that correspond to different splice variants of transcripts encoding human calcium channel subunits.

DNA encoding types and subtypes of each of the α_1, α_2, β and γ subunit of voltage-dependent human calcium channels has been cloned herein by screening human cDNA libraries prepared from isolated poly A+ mRNA from cell lines or tissue of human origin having such calcium channels. Among the sources of such cells or tissue for obtaining mRNA are human brain tissue or a human cell line of neural origin, such as a neuroblastoma cell line, human skeletal muscle or smooth muscle cells, and the like. Methods of preparing cDNA libraries are well known in the art [see generally Ausubel *et al.* (1987) *Current Protocols in Molecular Biology*, Wiley-Interscience, New York; and Davis *et al.* (1986) *Basic Methods in Molecular Biology*, Elsevier Science Publishing Co., New York].

With respect to each of the respective subunits of a human calcium channel (α_1, α_2, β or γ), once the DNA encoding the channel subunit was identified by a nucleic acid screening method, the isolated clone was used for further screening to identify overlapping clones. Some of the cloned DNA fragments can and have been subcloned into an appropriate vector such as pIBI24/25 (IBI, New Haven, CT), M13mp18/19, pGEM4, pGEM3, pGEM7Z, pSP72 and other such vectors known to those of skill in this art, and characterized by DNA sequencing and restriction enzyme mapping. A sequential series of overlapping clones may thus be generated for each of the subunits until a full-length clone can be prepared by methods, known to those of skill in the art, that include identification of translation initiation (start) and translation termination (stop) codons. For expression of the cloned DNA, the 5′ noncoding region and other transcriptional and translational control regions of such a clone may be replaced with an efficient ribosome binding site and other regulatory regions as known in the art. Examples II-VI, below, describe in detail the cloning of each of the various subunits of a human calcium channel as well as subtypes and splice variants, including tissue-specific variants thereof. In the instances in which partial sequences of a subunit are disclosed, it is well within the skill of the art, in view of the teaching herein, to obtain the corresponding full-length nucleotide sequence encoding the subunit, subtype or splice variant thereof.

Identification and isolation of DNA encoding α_1 subunits

A number of voltage-dependent calcium channel α_1 subunit genes, which are expressed in the human CNS, have been identified and have been designated as α_{1A}, α_{1B} (or VDCC IV), α_{1C} (or VDCC II) and α_{1D} (or VDCC III). DNA, isolated from a human neuronal cDNA library, that encodes each of the subunit types has been isolated. DNA encoding subtypes

of each of the types, which arise as splice variants are also provided. Subtypes are herein designated, for example, as α_{1B-1}, α_{1B-2}.

The α_1 subunits types A B, C, and D of voltage-dependent calcium channels, and subtypes thereof, differ with respect to sensitivity to known classes of calcium channel agonists and antagonists, such as DHPs, phenylalkylamines, omega conotoxin (ω-CgTx) and pyrazonoylguanidines. They also appear to differ in the holding potential and ion the kinetics of currents produced upon depolarization of cell membranes containing calcium channels that include different types of α_1 subunits.

DNA that encodes an α_1-subunit that binds to at least one compound selected from among dihydropyridines, phenylalkylamines, ω-CgTx, components of funnel web spider toxin, and pyrazonoylguanidines is provided. For example, the α_{1B} subunit provided herein appears to specifically interact with ω-CgTx in N-type channels, and the α_{1D} subunit provided herein specifically interacts with DHPs in L-type channels.

Identification and isolation of DNA encoding the α_{1D} human calcium channel subunit

The α_{1D} subunit cDNA has been isolated using fragments of the rabbit skeletal muscle calcium channel α_1 subunit cDNA as a probe to screen a cDNA library of a human neuroblastoma cell line, IMR32, to obtain clone α1.36. This clone was used as a probe to screen additional IMR32 cell cDNA libraries to obtain overlapping clones, which were then employed for screening until a sufficient series of clones to span the length of the nucleotide sequence encoding the human α_{1D} subunit were obtained. Full-length clones encoding α_{1D} were constructed by ligating portions of partial α_{1D} clones as described in Example II. SEQ ID No. 1 shows the 7,635 nucleotide sequence of the cDNA encoding the α_{1D} subunit. There is a 6,483 nucleotide sequence

reading frame which encodes a sequence of 2,161 amino acids (as set forth in SEQ ID No. 1).

SEQ ID No. 2 provides the sequence of an alternative exon encoding the IS6 transmembrane domain [see Tanabe, T., *et al.* (1987) *Nature 328*:313-318 for a description of transmembrane domain terminology] of the α_{1D} subunit.

SEQ ID No. 1 also shows the 2,161 amino acid sequence deduced from the human neuronal calcium channel α_{1D} subunit DNA. Based on the amino acid sequence, the α_{1D} protein has a calculated Mr of 245,163. The α_{1D} subunit of the calcium channel contains four putative internal repeated sequence regions. Four internally repeated regions represent 24 putative transmembrane segments, and the amino- and carboxyl-termini extend intracellularly.

The α_{1D} subunit has been shown to mediate DHP-sensitive, high-voltage-activated, long-lasting calcium channel activity. This calcium channel activity was detected when oöcytes were co-injected with RNA transcripts encoding an α_{1D} and β_1 or α_{1D}, α_2 and β_1 subunits. This activity was distinguished from Ba^{2+} currents detected when oöcytes were injected with RNA transcripts encoding the β_1 ± α_2 subunits. These currents pharmacologically and biophysically resembled Ca^{2+} currents reported for uninjected oöcytes.

Identification and isolation DNA encoding the α_{1A} human calcium channel subunit

Biological material containing DNA encoding the α_{1A} subunit had been deposited in the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under the terms of the Budapest Treaty on the International Recognition of Deposits of Microorganisms for Purposes of Patent Procedure and the Regulations promulgated under this Treaty. Samples of the deposited material are and will be available to industrial property offices and other persons legally entitled to receive them

under the terms of said Treaty and Regulations and otherwise in compliance with the patent laws and regulations of the United States of America and all other nations or international organizations in which this application, or an application claiming priority of this application, is filed or in which any patent granted on any such application is granted.

The α_{1A} subunit is encoded by an approximately 3 kb insert in λgt10 phage designated α1.254 in *E. coli* host strain NM514. A phage lysate of this material has been deposited as at the American Type Culture Collection under ATCC Accession No. , as described above. DNA encoding α_{1A} may also be identified by screening with a probe prepared from DNA that has SEQ ID No. 21:

5′ CTCAGTACCATCTCTGATACCAGCCCCA 3′.

Identification and isolation of DNA encoding the α_{1B} human calcium channel subunit

DNA encoding the α_{1B} subunit was isolated by screening a human basal ganglia cDNA library with fragments of the rabbit skeletal muscle calcium channel α_1 subunit-encoding cDNA. A portion of one of the positive clones was used to screen an IMR32 cell cDNA library. Clones that hybridized to the basal ganglia DNA probe were used to further screen an IMR32 cell cDNA library to identify overlapping clones that in turn were used to screen a human hippocampus cDNA library. In this way, a sufficient series of clones to span nearly the entire length of the nucleotide sequence encoding the human α_{1B} subunit was obtained. PCR amplification of specific regions of the IMR32 cell α_{1B} mRNA yielded additional segments of the α_{1B} coding sequence.

A full-length α_{1B} DNA clone was constructed by ligating portions of the partial cDNA clones as described in Example II.C. SEQ ID Nos. 7 and 8 show the nucleotide sequences of DNA clones encoding the α_{1B} subunit as well as the deduced amino acid sequences. The α_{1B} subunit encoded by SEQ ID No.

7 is referred to as the α_{1B-1} subunit to distinguish it from another α_{1B} subunit, α_{1B-2}, encoded by the nucleotide sequence shown as SEQ ID No. 8, which is derived from alternative splicing of the α_{1B} subunit transcript.

PCR amplification of IMR32 cell mRNA using oligonucleotide primers designed according to nucleotide sequences within the α_{1B-1}-encoding DNA has identified variants of the α_{1B} transcript that appear to be splice variants because they contain divergent coding sequences.

Identification and isolation of DNA encoding the α_{1C} human calcium channel subunit

Numerous α_{1C}-specific DNA clones were isolated. Characterization of the sequence revealed the α_{1C} coding sequence, the α_{1C} initiation of translation sequence, and an alternatively spliced region of α_{1C}. Alternatively spliced variants of the α_{1C} subunit have been identified. SEQ ID No. 3 sets forth DNA encoding an α_{1C} subunit. The DNA sequences set forth in SEQ ID No. 4 and No. 5 encode two possible amino terminal ends of the α_{1C} protein. SEQ ID No. 6 encodes an alternative exon for the IV S3 transmembrane domain.

The isolation and identification of DNA clones encoding portions of the α_{1C} subunit is described in detail in Example II.

DNA encoding other α_1 subunits, including α_{1A}, has also been isolated. Additional such subunits may also be isolated and identified using the DNA provided herein as described for the α_{1B}, α_{1C} and α_{1D} subunits or using other methods known to those of skill in the art.

Identification and isolation DNA encoding β human calcium channel subunits

DNA encoding β_1

To isolate DNA encoding the β_1 subunit, a human hippocampus cDNA library was screened by hybridization to a DNA fragment encoding a rabbit skeletal muscle calcium

channel β subunit. A hybridizing clone was selected and was in turn used to isolate overlapping clones until the overlapping clones encompassing DNA encoding the entire the human calcium channel β subunit were isolated and sequenced.

Five alternatively spliced forms of the human calcium channel β_1 subunit have been identified and DNA encoding a number of forms have been isolated. These forms are designated β_{1-1}, expressed in skeletal muscle, β_{1-2}, expressed in the CNS, β_{1-3}, also expressed in the in the CNS, β_{1-4}, expressed in aorta tissue and HEK 293 cells, and β_{1-5}, expressed in HEK 293 cells. A full-length DNA clone encoding the β_{1-2} subunit has been constructed. The subunits β_{1-1}, β_{1-2}, β_{1-4} and β_{1-5} have been identified by PCR analysis as alternatively spliced forms of the β subunit.

The alternatively spliced variants were identified by comparison of amino acid sequences encoded by the human neuronal and rabbit skeletal muscle calcium channel β subunit-encoding DNA. This comparison revealed a 45-amino acid deletion in the human β subunit compared to the rabbit β subunit. Using DNA from the region as a probe for DNA cloning, as well as PCR analysis and DNA sequencing of this area of sequence divergence, alternatively spliced forms of the human calcium channel β subunit transcript were identified. For example, the sequence of DNA encoding one splice variant β_{1-2} is set forth in SEQ ID No. 9. SEQ ID No. 10 sets forth the sequence of the β_{1-3} subunit (nt 1-1851, including 3′ untranslated sequence nt 1795-1851), which is another splice variant of the β subunit primary transcript. β_{1-2} and β_{1-3} are human neuronal β subunits. DNA distinctive for a portion of a β subunit (β_{1-4}) of a human aortic calcium channel and also human embryonic kidney (HEK) cells is set forth in SEQ ID No. 12 (nt 1-13 and 191-271). The sequence of DNA encoding a portion of a human calcium channel β

subunit expressed in skeletal muscle (β_{1-1}) is shown in SEQ ID No. 12 (nt 1-13 and 35-271).

DNA encoding β_3

DNA encoding the β_3 subunit and any splice variants thereof may be isolated by screening a library, as described above for the β_1 subunit, using DNA probes prepared according to SEQ ID Nos. 19 and 20 or using all or a portion of the deposited β_3 clone plasmid β1.42 (ATCC Accession No. 69048).

The *E. coli* host containing plasmid β1.42 that includes DNA encoding the β_3 subunit have been deposited as ATCC Accession No. 69048 in the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under the terms of the Budapest Treaty on the International Recognition of Deposits of Microorganisms for Purposes of Patent Procedure and the Regulations promulgated under this Treaty. Samples of the deposited material are and will be available to industrial property offices and other persons legally entitled to receive them under the terms of said Treaty and Regulations and otherwise in compliance with the patent laws and regulations of the United States of America and all other nations or international organizations in which this application, or an application claiming priority of this application, is filed or in which any patent granted on any such application is granted.

The β_3 encoding plasmid is designated β1.42. The plasmid contains a 2.5 kb *Eco*RI fragment encoding β_3 inserted into vector pGem®7zF(+) and has been deposited in *E. coli* host strain DN5α. A partial DNA sequence of the 5′ and 3′ ends of β_3 are set forth in SEQ ID Nos. 19 and 20, respectively.

Identification and isolation DNA encoding the α2 human calcium channel subunit

DNA encoding a human neuronal calcium channel α_2 subunit was isolated in a manner substantially similar to that used for isolating DNA encoding an α_1 subunit, except that a human genomic DNA library was probed under low and high stringency conditions with a fragment of DNA encoding the rabbit skeletal muscle calcium channel α_2 subunit. The fragment included nucleotides having a sequence corresponding to the nucleotide sequence between nucleotides 43 and 272 inclusive of rabbit back skeletal muscle calcium channel α_2 subunit cDNA as disclosed in PCT International Patent Application Publication No. WO 89/09834, which corresponds to U.S. Application Serial No. 07/620,520, which is a continuation-in-part of United States Serial No. 176,899, filed April 4, 1988, which applications have been incorporated herein by reference.

Example IV describes the isolation of DNA clones encoding α_2 subunits of a human calcium channel from a human DNA library using genomic DNA and cDNA clones, identified by hybridization to the genomic DNA, as probes.

SEQ ID No. 11 shows the sequence of DNA encoding an α_2 subunit. As described in Example V, PCR analysis of RNA from human skeletal muscle, brain tissue and aorta using oligonucleotide primers specific for a region of the human neuronal α_2 subunit cDNA that diverges from the rabbit skeletal muscle calcium channel α_2 subunit cDNA identified splice variants of the human calcium channel α_2 subunit transcript.

Identification and isolation of DNA encoding γ human calcium channel subunits

DNA encoding a human neuronal calcium channel γ subunit has been isolated as described in detail in Example VI. SEQ ID No. 14 shows the nucleotide sequence at the 3'-end of this DNA which includes a reading frame encoding a sequence of 43 amino acid residues.

Preparation of recombinant eukaryotic cells containing DNA encoding heterologous calcium channel subunits

DNA encoding one or more of the calcium channel subunits or a portion of a calcium channel subunit may be introduced into a host cell for expression or replication of the DNA. Such DNA may be introduced using methods described in the following examples or using other procedures well known to those skilled in the art. Incorporation of cloned DNA into a suitable expression vector, transfection of eukaryotic cells with a plasmid vector or a combination of plasmid vectors, each encoding one or more distinct genes or with linear DNA, and selection of transfected cells are also well known in the art [see, *e.g.*, Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor Laboratory Press].

Cloned full-length DNA encoding any of the subunits of a human calcium channel may be introduced into a plasmid vector for expression in a eukaryotic cell. Such DNA may be genomic DNA or cDNA. Host cells may be transfected with one or a combination of said plasmids, each of which encodes at least one calcium channel subunit. Alternatively, host cells may be transfected with linear DNA using methods well known to those of skill in the art.

While the DNA provided herein may be expressed in any eukaryotic cell, including yeast cells such as *P. pastoris* [see, *e.g.*, Cregg *et al.* (1987) *Bio/Technology* 5:479], mammalian expression systems for expression of the DNA encoding the human calcium channel subunits provided herein are preferred.

The heterologous DNA may be introduced by any method known to those of skill in the art, such as transfection with a vector encoding the heterologous DNA. Particularly preferred vectors for transfection of mammalian cells are the pSV2dhfr expression vectors, which contain the SV40 early promoter, mouse dhfr gene, SV40 polyadenylation and

splice sites and sequences necessary for maintaining the vector in bacteria, cytomegalovirus (CMV) promoter-based vectors such as pCMV or pCDNA1, and MMTV promoter-based vectors. DNA encoding the human calcium channel subunits has been inserted in the vector pCDNA1 at a position immediately following the CMV promoter.

Stably or transiently transfected mammalian cells may be prepared by methods known in the art by transfecting cells with an expression vector having a selectable marker gene such as the gene for thymidine kinase, dihydrofolate reductase, neomycin resistance or the like, and, for transient transfection, growing the transfected cells under conditions selective for cells expressing the marker gene. Functional voltage-dependent calcium channels have been produced in HEK 293 cells transfected with a derivative of the vector pCDNA1 that contains DNA encoding a human calcium channel subunit.

The heterologous DNA may be maintained in the cell as an episomal element or may be integrated into chromosomal DNA of the cell. The resulting recombinant cells may then be cultured or subcultured (or passaged, in the case of mammalian cells) from such a culture or a subculture thereof. Methods for transfection, injection and culturing recombinant cells are known to the skilled artisan.

Eukaryotic cells in which DNA or RNA may be introduced, include any cells that are transfectable by such DNA or RNA or into which such DNA may be injected. Virtually any eukaryotic cell can serve as a vehicle for heterologous DNA. Preferred cells are those that can also express the DNA and RNA and most preferred cells are those that can form recombinant or heterologous calcium channels that include one or more subunits encoded by the heterologous DNA. Such cells may be identified empirically or selected from among those known to be readily transfected or injected. Preferred cells for introducing DNA include

those that can be transiently or stably transfected and include, but are not limited to cells of mammalian origin, such as COS cells, mouse L cells, CHO cells, human embryonic kidney cells, African green monkey cells and other such cells known to those of skill in the art, amphibian cells, such as *Xenopus laevis* oöcytes, or those of yeast such as *Saccharomyces cerevisiae* or *Pichia pastoris*. Preferred cells for expressing injected RNA transcripts include *Xenopus laevis* oöcytes. Cells that are preferred for transfection of DNA are those that can be readily and efficiently transfected. Such cells are known to those of skill in the art or may be empirically identified. Preferred cells include DG44 cells and HEK 293 cells, particularly HEK 293 cells that have been adapted for growth in suspension and that can be frozen in liquid nitrogen and then thawed and regrown. Such HEK 293 cells are described, for example in U.S. Patent No. 5,024,939 to Gorman [see, also Stillman *et al.* (1985) *Mol. Cell.Biol.* 5:2051-2060].

The cells may be used as vehicles for replicating heterologous DNA introduced therein or for expressing the heterologous DNA introduced therein. In certain embodiments, the cells are used as vehicles for expressing the heterologous DNA as a means to produce substantially pure human calcium channel subunits or heterologous calcium channels. Host cells containing the heterologous DNA may be cultured under conditions whereby the calcium channels are expressed. The calcium channel subunits may be purified using protein purification methods known to those of skill in the art. For example, antibodies, such as those provided herein, that specifically bind to one or more of the subunits may be used for affinity purification of the subunit or calcium channels containing the subunits.

Substantially pure subunits of a human calcium channel α_1 subunits of a human calcium channel, α_2 subunits of a

human calcium channel, β subunits of a human calcium channel and γ subunits of a human calcium channel are provided. Substantially pure isolated calcium channels that contain at least one of the human calcium channel subunits are also provided. Substantially pure calcium channels that contain a mixture of one or more subunits encoded by the host cell and one or more subunits encoded by heterologous DNA or RNA that has been introduced into the cell are also provided. Substantially pure subtype- or tissue-type specific calcium channels are also provided.

In other embodiments, eukaryotic cells that contain heterologous DNA encoding at least one of an α_1 subunit of a human calcium channel , an α_2 subunit of a human calcium channel, a β subunit of a human calcium channel and a γ subunit of a human calcium channel are provided. In accordance with one preferred embodiment, the heterologous DNA is expressed in the eukaryotic cell and preferably encodes a human calcium channel α_1 subunit.

In particularly preferred aspects, the eukaryotic cell which contains the heterologous DNA expresses it and forms a recombinant functional calcium channel activity. In more preferred aspects, the recombinant calcium channel activity is readily detectable because it is a type that is absent from the untransfected host cell or is of a magnitude not exhibited in the untransfected cell.

Preferred among such cells is a recombinant eukaryotic cell with a functional heterologous calcium channel. The recombinant cell can be produced by introduction of and expression of heterologous DNA or RNA transcripts encoding an α_1 subunit of a human calcium channel, more preferably also expressing, a heterologous DNA encoding a β subunit of a human calcium channel and/or heterologous DNA encoding an α_2 subunit of a human calcium channel. Especially preferred is the expression in such a recombinant cell of each of the α_1, β and α_2 subunits encoded by such heterologous DNA or

RNA transcripts, and optionally expression of heterologous DNA or an RNA transcript encoding a γ subunit of a human calcium channel.

In certain embodiments, the eukaryotic cell with a heterologous calcium channel is produced by introducing into the cell a first composition, which contains at least one RNA transcript that is translated in the cell into a subunit of a human calcium channel. In preferred embodiments, the subunits that are translated include an α_1 subunit of a human calcium channel. More preferably, the composition that is introduced contains an RNA transcript which encodes an α_1 subunit of a human calcium channel and also contains (1) an RNA transcript which encodes a β subunit of a human calcium channel and/or (2) an RNA transcript which encodes an α_2 subunit of a human calcium channel. Especially preferred is the introduction of RNA encoding an α_1, a β and an α_2 human calcium channel subunit, and, optionally, a γ subunit of a human calcium channel.

Methods for *in vitro* transcription of a cloned DNA and injection of the resulting RNA into eukaryotic cells are well known in the art. Transcripts of any of the full-length DNA encoding any of the subunits of a human calcium channel may be injected alone or in combination with other transcripts into eukaryotic cells for expression in the cells. Amphibian oöcytes are particularly preferred for expression of *in vitro* transcripts of the human calcium channel subunit cDNA clones provided herein.

The functional calcium channels may preferably include at least an α_1 subunit and a β subunit of a human calcium channel. Eukaryotic cells expressing these two subunits and also cells expressing additional subunits, have been prepared by transfection of DNA and by injection of RNA transcripts. Such cells have exhibited voltage-dependent calcium channel activity attributable to calcium channels that contain one or more of the heterologous human calcium

channel subunits. For example, eukaryotic cells expressing heterologous calcium channels containing an α_2 subunit in addition to the α_1 subunit and a β subunit have been shown to exhibit increased calcium selective ion flow across the cellular membrane in response to depolarization, indicating that the α_2 subunit may potentiate calcium channel function.

Eukaryotic cells which express heterologous calcium channels containing at least a human α_1 subunit, a human β subunit and a human α_2 subunit are preferred. Eukaryotic cells transformed with a composition containing cDNA or an RNA transcript that encodes an α_1 subunit alone or in combination with a β and/or an α_2 subunit may be used to produce cells that express functional calcium channels. Since recombinant cells expressing human calcium channels containing all of the of the human subunits encoded by the heterologous cDNA or RNA are especially preferred, it is desirable to inject or transfect such host cells with a sufficient concentration of the subunit-encoding nucleic acids to form calcium channels that contain the human subunits encoded by heterologous DNA or RNA. The precise amounts and ratios of DNA or RNA encoding the subunits may be empirically determined and optimized for a particular combination of subunits, cells and assay conditions.

With respect to measurement of the activity of functional heterologous calcium channels, preferably, endogenous ion channel activity and, if desired, heterologous channel activity of channels that do not contain the desired subunits, of a host cell can be inhibited to a significant extent by chemical, pharmacological and electrophysiological means, including the use of differential holding potential, to increase the S/N ratio of the measured heterologous calcium channel activity.

Among the uses for eukaryotic cells which recombinantly express one or more subunits are assays for

determining whether a test compound has calcium channel agonist or antagonist activity. Desirably, a host cell for the expression of calcium channel subunits does not produce endogenous calcium channel subunits of the type or in an amount that substantially interferes with the detection of heterologous calcium channel subunits in ligand binding assays or detection of heterologous calcium channel function, such as generation of calcium current, in functional assays.

With respect to ligand binding assays, the host cells preferably should not produce endogenous calcium channels which detectably interact with compounds having, at physiological concentrations (generally nanomolar or picomolar concentrations), affinity for calcium channels that contain one or all of the human calcium channel subunits provided herein.

With respect to ligand binding assays for identifying a compound which has affinity for calcium channels, cells are employed which express, preferably, at least a heterologous α_1 subunit. Transfected eukaryotic cells which express at least an α_1 subunit may be used to determine the ability of a test compound to specifically alter the activity of a calcium channel. Such ligand binding assays may be performed on intact transfected cells or membranes prepared therefrom.

The capacity of a test compound to bind to or otherwise interact with membranes that contain heterologous calcium channels or subunits thereof may be determined by using any appropriate method, such as competitive binding analysis, such as Scatchard plots, in which the binding capacity of such membranes is determined in the presence and absence of one or more concentrations of a compound having known affinity for the calcium channel. Where necessary, the results may be compared to a control experiment designed in accordance with methods known to

those of skill in the art. For example, as a negative control, the results may be compared to those of assays of an identically treated membrane preparation from host cells which have not been transfected with one or more subunit-encoding nucleic acids.

Stably or transiently transfected cells or injected cells which express voltage-dependent human calcium channels containing one or more of the subunits of a human calcium channel desirably may be used in assays to identify agents, such as calcium channel agonists and antagonists, that modulate calcium channel activity. Functionally testing the activity of test compounds, including compounds having unknown activity, for calcium channel agonist or antagonist activity to determine if the test compound potentiates, inhibits or otherwise alters the flow of calcium through a human calcium channel can be accomplished by (a) maintaining a eukaryotic cell which is transfected or injected to express a heterologous functional calcium channel capable of regulating the flow of calcium channel selective ions into the cell in a medium containing calcium channel selective ions (i) in the presence of and (ii) in the absence of a test compound; (b) maintaining the cell under conditions such that the heterologous calcium channels are substantially closed and endogenous calcium channels of the cell are substantially inhibited (c) depolarizing the membrane of the cell maintained in step (b) to an extent and for an amount of time sufficient to cause (preferably, substantially only) the heterologous calcium channels to become permeable to the calcium channel selective ions; and (d) comparing the amount and duration of current flow into the cell in the presence of the test compound to that of the current flow into the cell, or a substantially similar cell, in the absence of the test compound.

Functional recombinant or heterologous calcium channels may be identified by any method known to those of skill in the art. For example, electrophysiological procedures for measuring the current across an ion-selective membrane of a cell, which are well known, may be used. The amount and duration of the flow of calcium selective ions through heterologous calcium channels of a recombinant cell containing DNA encoding one or more of the subunits provided herein has been measured using electrophysiological recordings using a two electrode and the whole-cell patch clamp techniques. In order to improve the sensitivity of the assays, known methods can be used to eliminate or reduce non-calcium currents and calcium currents resulting from endogenous calcium channels, when measuring calcium currents through recombinant channels. For example, the DHP Bay K 8644 specifically enhances L-type calcium channel function by increasing the duration of the open state of the channels [see, *e.g.*, Hess, J.B., *et al.* (1984) *Nature 311*:538-544]. Prolonged opening of the channels results in calcium currents of increased magnitude and duration. Tail currents can be observed upon repolarization of the cell membrane after activation of ion channels by a depolarizing voltage command. The opened channels require a finite time to close or "deactivate" upon repolarization, and the current that flows through the channels during this period is referred to as a tail current. Because Bay K 8644 prolongs opening events in calcium channels, it tends to prolong these tail currents and make them more pronounced.

EXAMPLES

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLE I: PREPARATION OF LIBRARIES USED FOR ISOLATION OF DNA ENCODING HUMAN NEURONAL VOLTAGE-DEPENDENT CALCIUM CHANNEL SUBUNITS

A. RNA Isolation

1. IMR32 cells

IMR32 cells were obtained from the American Type Culture Collection (ATCC Accession No. CCL127, Rockville, MD) and grown in DMEM, 10% fetal bovine serum, 1% penicillin/streptomycin (GIBCO, Grand Island, NY) plus 1.0 mM dibutyryl cAMP (dbcAMP) for ten days. Total RNA was isolated from the cells according to the procedure described by H.C. Birnboim [(1988) *Nucleic Acids Research 16*:1487-1497]. Poly(A^+) RNA was selected according to standard procedures [see, *e.g.*, Sambrook *et al.* (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press; pg. 7.26-7.29].

2. Human thalamus tissue

Human thalamus tissue (2.34 g), obtained from the National Neurological Research Bank, Los Angeles, CA, that had been stored frozen at -70°C was pulverized using a mortar and pestle in the presence of liquid nitrogen and the cells were lysed in 12 ml of lysis buffer (5 M guanidinium isothiocyanate, 50 mM TRIS, pH 7.4, 10 mM EDTA, 5% β-mercaptoethanol). Lysis buffer was added to the lysate to yield a final volume of 17 ml. N-laurylsarcosine and CsCl were added to the mixture to yield final concentrations of 4% and 0.01 g/ml, respectively, in a final volume of 18 ml.

The sample was centrifuged at 9,000 rpm in a Sorvall SS34 rotor for 10 min at room temperature to remove the insoluble material as a pellet. The supernatant was divided into two equal portions and each was layered onto a 2-ml cushion of a solution of 5.7 M CsCl, 0.1 M EDTA contained in separate centrifuge tubes to yield approximately 9 ml per tube. The samples were centrifuged in an SW41 rotor at 37,000 rpm for 24 h at 20°C.

After centrifugation, each RNA pellet was resuspended in 3 ml ETS (10 mM TRIS, pH 7.4, 10 mM EDTA, 0.2% SDS) and combined into a single tube. The RNA was precipitated with 0.25 M NaCl and two volumes of 95% ethanol.

The precipitate was collected by centrifugation and resuspended in 4 ml PK buffer (0.05 M TRIS, pH 8.4, 0.14 M NaCl, 0.01 M EDTA, 1% SDS). Proteinase K was added to the sample to a final concentration of 200 μg/ml. The sample was incubated at 22°C for 1 h, followed by extraction with an equal volume of phenol:chloroform:isoamylalcohol (50:48:2) two times, followed by one extraction with an equal volume of chloroform: isoamylalcohol (24:1). The RNA was precipitated with ethanol and NaCl. The precipitate was resuspended in 400 μl of ETS buffer. The yield of total RNA was approximately 1.0 mg. Poly A^+ RNA (30 μg) was isolated from the total RNA according to standard methods as stated in Example I.A.1.

B. Library Construction

Double-stranded cDNA was synthesized according to standard methods [see, *e.g.*, Sambrook *et al.* (1989) IN: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Chapter 8]. Each library was prepared in substantially the same manner except for differences in: 1) the oligonucleotide used to prime the first strand cDNA synthesis, 2) the adapters that were attached to the double-stranded cDNA, 3) the method used to remove the free or unused adapters, and 4) the size of the fractionated cDNA ligated into the λ phage vector.

1. IMR32 cDNA library #1

Single-stranded cDNA was synthesized using IMR32 poly(A^+) RNA (Example I.A.1.) as a template and was primed using oligo $(dT)_{12-18}$ (Collaborative Research Inc., Bedford, MA). The single-stranded cDNA was converted to double-stranded cDNA and the yield was approximately 2μg. *Eco*I adapters:

5'-AATTCGGTACGTACACTCGAGC-3' = 22-mer (SEQ ID No.15)

3'- GCCATGCATGTGAGCTCG-5' = 18-mer (SEQ ID No.16)

also containing *Sna*BI and *Xho*I restriction sites were then added to the double-stranded cDNA according to the following procedure.

a. Phosphorylation of 18-mer

The 18-mer was phosphorylated using standard methods [see, e.g., Sambrook *et al.* (1989) IN: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Chapter 8] by combining in a 10 μl total volume the 18 mer (225 pmoles) with [^{32}P]γ-ATP (7000 Ci/mmole; 1.0 μl) and kinase (2 U) and incubating at 37° C for 15 minutes. After incubation, 1 μL 10 mM ATP and an additional 2 U of kinase were added and incubated at 37°C for 15 minutes. Kinase was then inactivated by boiling for 10 minutes.

b. Hybridization of 22-mer

The 22-mer was hybridized to the phosphorylated 18-mer by addition of 225 pmoles of the 22-mer (plus water to bring volume to 15 μl), and incubation at 65°C for 5 minutes. The reaction was then allowed to slow cool to room temperature.

The adapters were thus present at a concentration of 15 pmoles/μl, and were ready for cDNA-adapter ligation.

c. Ligation of adapters to cDNA

After the *Eco*RI, *Sna*BI, *Xho*I adapters were ligated to the double-stranded cDNA using a standard protocol [see, e.g., Sambrook *et al.* (1989) IN: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Chapter 8], the ligase was inactivated by heating the mixture to 72°C for 15 minutes. The following reagents were added to the cDNA ligation reaction and heated at 37°C for 30 minutes: cDNA ligation reaction (20 μl), water (24 μl), 10x kinase buffer (3 μl), 10 mM ATP (1 μl) and kinase (2μl of 2 U/μl). The reaction was stopped by the addition

of 2 µl 0.5M EDTA, followed by one phenol/chloroform extraction and one chloroform extraction.

d. Size Selection and Packaging of cDNA

The double-stranded cDNA with the *Eco*RI, *Sna*BI, *Xho*I adapters ligated was purified away from the free or unligated adapters using a 5 ml Sepharose CL-4B column (Sigma, St. Louis, MO). 100 µl fractions were collected and those containing the cDNA, determined by monitoring the radioactivity, were pooled, ethanol precipitated, resuspended in TE buffer and loaded onto a 1% agarose gel. After the electrophoresis, the gel was stained with ethidium bromide and the 1 to 3 kb fraction was cut from the gel. The cDNA embedded in the agarose was eluted using the "Geneluter Electroelution System" (Invitrogen, San Diego, CA). The eluted cDNA was collected by ethanol precipitation and resuspended in TE buffer at 0.10 pmol/µl. The cDNA was ligated to 1 µg of *Eco*RI digested, dephosphorylated λgt11 in a 5 µl reaction volume at a 2- to 4- fold molar excess ratio of cDNA over the λgt11 vector. The ligated λgt11 containing the cDNA insert was packaged into λ phage virions *in vitro* using the Gigapack (Stratagene, La Jolla, CA) kit. The packaged phage were plated on an *E. coli* Y1088 bacterial lawn in preparation for screening.

2. IMR32 cDNA library #2

This library was prepared as described (Example I.B.1.) with the exception that 3 to 9 kb cDNA fragments were ligated into the λgt11 phage vector rather than the 1 to 3 kb fragments.

3. IMR32 cDNA library #3

IMR32 cell poly(A^+) RNA (Example I.A.1.) was used as a template to synthesize single-stranded cDNA. The primers for the first strand cDNA synthesis were random primers (hexadeoxy-nucleotides [$pd(N)_6$] Cat #5020-1, Clontech, Palo Alto, CA). The double-stranded cDNA was synthesized

(Example I.B.1.), *Eco*RI, *Sna*BI, *Xho*I adapters were added to the cDNA (Example I.B.1.), the unligated adapters were removed (Example I.B.1.), and the double-stranded cDNA with the ligated adapters was fractionated on an agarose gel (Example I.B.1.). The cDNA fraction greater than 1.8 kb was eluted from the agarose (Example I.B.1.), ligated into λgt11, packaged, and plated into a bacterial lawn of Y1088 (Example I.B.1.).

4. IMR32 cDNA library #4

IMR32 cell poly(A^+) RNA (Example I.A.1.) was used as a template to synthesize single-stranded cDNA. The primers for the first strand cDNA synthesis were oligonucleotides: 89-365a specific for the α_{1D} (VDCC III) type α_1-subunit (see Example II.A.) coding sequence (the complementary sequence of nt 2927 to 2956, SEQ ID No. 1), 89-495 specific for the α_{1C} (VDCC II) type α_1-subunit (see Example II.B.) coding sequence (the complementary sequence of nt 852 to 873, SEQ ID No. 3), and 90-12 specific for the α_{1C}-subunit coding sequence (the complementary sequence of nt 2496 to 2520, SEQ ID No. 3). The cDNA library was then constructed as described (Example I.B.3), except that the cDNA size-fraction greater than 1.5 kb was eluted from the agarose rather than the greater than 1.8 kb fraction.

5. IMR32 cDNA library #5

The cDNA library was constructed as described (Example I.B.3.) with the exception that the size-fraction greater than 1.2 kb was eluted from the agarose rather than the greater than 1.8 kb fraction.

6. Human thalamus cDNA library #6

Human thalamus poly (A^+) RNA (Example I.A.2.) was used as a template to synthesize single-stranded cDNA. Oligo (dT) was used to prime the first strand synthesis (Example I.B.1.). The double-stranded cDNA was synthesized (Example I.B.1.) and *Eco*RI, *Kpn*I, *Nco*I adapters of the following sequence:

5′ CCATGGTACCTTCGTTGACG 3′= 20 mer (SEQ ID NO. 17)
3′ GGTACCATGGAAGCAACTGCTTAA 5′= 24 mer (SEQ ID NO. 18)
were ligated to the double-stranded cDNA as described (Example I.B.1.) with the 20-mer replacing the 18-mer and the 24-mer replacing the 22-mer. The unligated adapters were removed by passing the cDNA-adapter mixture through a 1 ml Bio Gel A-50 (Bio-Rad Laboratories, Richmond, CA.) column. Fractions (30 µl) were collected and 1 µl of each fraction in the first peak of radioactivity was electrophoresed on a 1% agarose gel. After electrophoresis, the gel was dried on a vacuum gel drier and exposed to x-ray film. The fractions containing cDNA fragments greater than 600 bp were pooled, ethanol precipitated, and ligated into λgt11 (Example I.B.1.). The construction of the cDNA library was completed as described (Example I.B.1.).

C. Hybridization and Washing Conditions

Hybridization of radiolabelled nucleic acids to immobilized DNA for the purpose of screening cDNA libraries, DNA Southern transfers, or northern transfers was routinely performed in standard hybridization conditions [5 x SSPE, 5x Denhardt's, 50% deionized formamide, 200 µg/ml sonicated herring sperm DNA (Cat #223646, Boehringer Mannheim Biochemicals, Indianapolis, IN)]. The recipes for SSPE and Denhardt's and the preparation of deionized formamide are described, for example, in Sambrook *et al.* (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Chapter 8). In some hybridizations, lower stringency conditions were used in that 10% deionized formamide replaced 50% deionized formamide described for the standard hybridization conditions.

The washing conditions for removing the non-specific probe from the filters was either high, medium, or low stringency as described below:

1) high stringency: 0.1 x SSPE, 0.1% SDS, 65°C
2) medium stringency: 0.2 x SSPE, 0.1% SDS, 50°C
3) low stringency: 1.0 x SSPE, 0.1% SDS, 50°C.

It is understood that equivalent stringencies may be achieved using alternative buffers, salts and temperatures.

EXAMPLE II: ISOLATION OF DNA ENCODING THE HUMAN NEURONAL CALCIUM CHANNEL α_1 SUBUNIT

A. Isolation of DNA encoding the α_{1D} subunit

1. Reference list of partial α_{1D} cDNA clones

Numerous α_{1D}-specific cDNA clones were isolated in order to characterize the complete α_{1D} coding sequence plus portions of the 5′ and 3′ untranslated sequences. SEQ ID No. 1 shows the complete α_{1D} DNA coding sequence, plus 510 nucleotides of α_{1D} 5′ untranslated sequence ending in the guanidine nucleotide adjacent to the adenine nucleotide of the proposed initiation of translation as well as 642 nucleotides of 3′ untranslated sequence. Also shown in SEQ ID No. 1 is the deduced amino acid sequence. A list of partial cDNA clones used to characterize the α_{1D} sequence and the nucleotide position of each clone relative to the full-length α_{1D} cDNA sequence, which is set forth in SEQ ID No. 1, is shown below. The isolation and characterization of these clones are described below (Example II.A.2.).

IMR32	1.144	nt. 1 to 510 of 5′ untranslated sequence,	SEQ ID No. 1
		nt. 511 to 2431,	SEQ ID No. 1
IMR32*	1.136	nt. 1627 to 2988,	SEQ ID No. 1
		nt. 1 to 104 of additional exon,	SEQ ID No. 2
IMR32@	1.80	nt. 2083 to 6468,	SEQ ID No. 1
IMR32#	1.36	nt. 2857 to 4281,	SEQ ID No. 1
IMR32	1.163	nt. 5200 to 7635,	SEQ ID No. 1

* 5′ of nt 1627, IMR32 1.136 encodes an intron and an additional exon described in Example II.A.2.d.

@ IMR32 1.80 contains two deletions, nt 2984 to 3131 and nt 5303 to 5349 (SEQ ID No. 1). The 148 nt deletion (nt. 2984 to 3131) was corrected by performing a polymerase chain reaction described in Example II.A.3.b.

IMR32 1.36 contains a 132 nt deletion (nt. 3081 to 3212).

2. Isolation and characterization of individual clones listed in Example II.A.1.

a. IMR32 1.36

Two million recombinants of the IMR32 cDNA library #1 (Example I.B.1.) were screened in duplicate at a density of approximately 200,000 plaques per 150 mm plate using a mixture of radiolabelled fragments of the coding region of the rabbit skeletal muscle calcium channel α_1 cDNA [for the sequence of the rabbit skeletal muscle calcium channel α_1 subunit cDNA, see, Tanabe *et al.* (1987). *Nature* 328:313-318]:

Fragment	Nucleotides
*Kpn*I-*Eco*RI	-78 to 1006
*Eco*RI-*Xho*I	1006 to 2653
*Apa*I-*Apa*I	3093 to 4182
*Bgl*II-*Sac*I	4487 to 5310

The hybridization was performed using low stringency hybridization conditions (Example I.C.) and the filters were washed under low stringency (Example I.C.). Only one α_{1D}-specific recombinant (IMR32 1.36) of the 2 x 10^6 screened was identified. IMR32 1.36 was plaque purified by standard methods (J. Sambrook *et al.* (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Chapter 8) subcloned into pGEM3 (Promega, Madison, WI) and characterized by DNA sequencing.

b. IMR32 1.80

Approximately 1 x 10^6 recombinants of the IMR32 cDNA library #2 (Example I.B.2.) were screened in duplicate at a density of approximately 100,000 plaques per 150 mm plate using the IMR32 1.36 cDNA fragment (Example II.A.1) as a

probe. Standard hybridization conditions were used (Example I.C), and the filters were washed under high stringency (Example I.C.). Three positive plaques were identified one of which was IMR32 1.80. IMR32 1.80 was plaque purified by standard methods, restriction mapped, subcloned, and characterized by DNA sequencing.

c. IMR32 1.144

Approximately 1 x 10^6 recombinants of the IMR32 cDNA library #3 (Example I.B.3) were screened with the *Eco*RI-*Pvu*II fragment (nt 2083 to 2518, SEQ ID No. 1) of IMR32 1.80. The hybridization was performed using standard hybridization conditions (Example I.C.) and the filters were washed under high stringency (Example I.C.). Three positive plaques were identified one of which was IMR32 1.144. IMR32 1.144 was plaque purified, restriction mapped, and the cDNA insert was subcloned into pGEM7Z (Promega, Madison, WI) and characterized by DNA sequencing. This characterization revealed that IMR32 1.144 has a series of ATG codons encoding seven possible initiating methionines (nt 511 to 531, SEQ ID No. 1). PCR analysis, and DNA sequencing of cloned PCR products encoding these seven ATG codons confirmed that this sequence is present in the α_{1D} transcript expressed in dbcAMP-induced IMR32 cells.

d. IMR32 1.136

Approximately 1 x 10^6 recombinants of the IMR32 cDNA library #4 (Example I.B.4) were screened with the *Eco*RI-*Pvu*II fragment (nt 2083 to 2518, SEQ ID No. 1) of IMR32 1.80 (Example II.A.1.). The hybridization was performed using standard hybridization conditions (Example I.C.) and the filters were washed under high stringency (Example I.C.). Six positive plaques were identified one of which was IMR32 1.136. IMR32 1.136 was plaque purified, restriction mapped, and the cDNA insert was subcloned into a standard plasmid vector, pSP72 (Promega, Madison, WI.), and characterized by DNA sequencing. This characterization

revealed that IMR32 1.136 encodes an incompletely spliced α_{1D} transcript. The clone contains nucleotides 1627 to 2988 of SEQ ID No. 1 preceded by an approximate 640 bp intron. This intron is then preceded by a 104 nt exon (SEQ ID No. 2) which is an alternative exon encoding the IS6 transmembrane domain [see, *e.g.*, Tanabe *et al.* (1987) *Nature 328*:313-318 for a description of the IS1 to IVS6 transmembrane terminology] of the α_{1D} subunit and can replace nt 1627 to 1730, SEQ ID No. 1, to produce a completely spliced α_{1D} transcript.

e. IMR32 1.163

Approximately 1 x 10^6 recombinants of the IMR32 cDNA library #3 (Example I.B.3.) were screened with the *NcoI-XhoI* fragment of IMR32 1.80 (Example II.A.1.) containing nt 5811 to 6468 (SEQ ID No. 1). The hybridization was performed using standard hybridization conditions (Example I.C.) and the filters were washed under high stringency (Example I.C.). Three positive plaques were identified one of which was IMR32 1.163. IMR32 1.163 was plaque purified, restriction mapped, and the cDNA insert was subcloned into a standard plasmid vector, pSP72 (Promega, Madison, WI.), and characterized by DNA sequencing. This characterization revealed that IMR32 1.163 contains the α_{1D} termination codon, nt 6994 to 6996 (SEQ ID No. 1).

3. Construction of a full-length α_{1D} cDNA [pVDCCIII(A)]

α_{1D} cDNA clones IMR32 1.144, IMR32 1.136, IMR32 1.80, and IMR32 1.163 (Example II.A.2.) overlap and include the entire α_{1D} coding sequence, nt 511 to 6993 (SEQ ID No. 1), with the exception of a 148 bp deletion, nt 2984 to 3131 (SEQ ID No. 1). Portions of these partial cDNA clones were ligated to generate a full-length α_{1D} cDNA in a eukaryotic expression vector. The resulting vector was called pVDCCIII(A). The construction of pVDCCIII(A) was performed in four steps described in detail below: (1) the

construction of pVDCCIII/5′ using portions of IMR32 1.144, IMR32 1.136, and IMR32 1.80, (2) the construction of pVDCCIII/5′.3 that corrects the 148 nt deletion in the IMR32 1.80 portion of pVDCCIII/5′, (3) the construction of pVDCCIII/3′.1 using portions of IMR32 1.80 and IMR32 1.163, and (4) the ligation of a portion of the pVDCCIII/5′.3 insert, the insert of pVDCCIII/3′.1, and pcDNA1 (Invitrogen, San Diego, CA) to form pVDCCIII(A). The vector pcDNA1 is a eukaryotic expression vector containing a cytomegalovirus (CMV) promoter which is a constitutive promoter recognized by mammalian host cell RNA polymerase II.

Each of the DNA fragments used in preparing the full-length construct was purified by electrophoresis through an agarose gel onto DE81 filter paper (Whatman, Clifton, NJ) and elution from the filter paper using 1.0 M NaCl, 10 mM TRIS, pH 8.0, 1 mM EDTA. The ligations typically were performed in a 10 μl reaction volume with an equal molar ratio of insert fragment and a two-fold molar excess of the total insert relative to the vector. The amount of DNA used was normally about 50 ng to 100 ng.

a. pVDCCIII/5′

To construct pVDCCIII/5′, IMR32 1.144 (Example II.A.2.c.) was digested with *Xho*I and *Eco*RI and the fragment containing the vector (pGEM7Z), α_{ID} nt 1 to 510 (SEQ ID No. 1), and α_{ID} nt 511 to 1732 (SEQ ID No. 1) was isolated by gel electrophoresis. The *Eco*RI-*Apa*I fragment of IMR32 1.136 (Example II.A.2.d.) nucleotides 1732 to 2667 (SEQ ID No. 1) was isolated, and the *Apa*I-*Hin*dIII fragment of IMR32 1.80 (Example II.A.2.b.), nucleotides 2667 to 4492 (SEQ ID No. 1) was isolated. The three DNA clones were ligated to form pVDCCIII/5′ containing nt 1 to 510 (5′ untranslated sequence; SEQ ID No. 1) and nt 511 to 4492 (SEQ ID No. 1).

b. pVDCCIII/5'.3

Comparison of the IMR32 1.36 and IMR32 1.80 DNA sequences revealed that these two cDNA clones differ through the α_{1D} coding sequence, nucleotides 2984 to 3212. PCR analysis of IMR32 1.80 and dbcAMP-induced (1.0 mM, 10 days) IMR32 cytoplasmic RNA (isolated according to Ausubel, F.M. *et al.* (Eds) (1988) *Current Protocols in Molecular Biology*, John Wiley and Sons, New York) revealed that IMR32 1.80 had a 148 nt deletion, nt 2984 to 3131 (SEQ ID No. 1), and that IMR32 1.36 had a 132 nt deletion, nt 3081 to 3212. To perform the PCR analysis, amplification was primed with α_{1D}-specific oligonucleotides 112 (nt 2548 to 2572, SEQ ID No. 1) and 311 (the complementary sequence of nt 3928 to 3957, SEQ ID No. 1). These products were then reamplified using α_{1D}-specific oligonucleotides 310 (nt 2583 to 2608 SEQ ID No. 1) and 312 (the complementary sequence of nt 3883 to 3909). This reamplified product, which contains *Acc*I and *Bgl*II restriction sites, was digested with *Acc*I and *Bgl*II and the *Acc*I-*Bgl*II fragment, nt 2764 to 3890 (SEQ ID No. 1) was cloned into *Acc*I-*Bgl*II digested pVDCCIII/5' to replace the *Acc*I-*Bgl*II pVDCCIII/5' fragment that had the deletion. This new construct was named pVDCCIII/5'.3. DNA sequence determination of pVDCCIII/5'.3 through the amplified region confirmed the 148 nt deletion in IMR32 1.80.

c. pVDCCIII/3'.1

To construct pVDCCIII/3'.1, the cDNA insert of IMR32 1.163 (Example II.A.2.e.) was subcloned into pBluescript II (Stratagene, La Jolla, CA) as an *Xho*I fragment. The *Xho*I sites on the cDNA fragment were furnished by the adapters used to construct the cDNA library (I.B.3.). The insert was oriented such that the translational orientation of the insert of IMR32 1.163 was opposite to that of the *lacZ* gene present in the plasmid, as confirmed by analysis of restriction enzyme digests of the resulting plasmid. This was done to preclude the possibility of expression of α_{1D}

encoding two lysine residues, were deleted from pVDCCIII(A) and replaced with an efficient ribosomal binding site (5′-ACCACC-3′) to form pVDCCIII.RBS(A). Expression experiments in which transcripts of this construct were injected into *Xenopus laevis* oöcytes did not result in an enhancement in the recombinant voltage-dependent calcium channel expression level relative to the level of expression in oöcytes injected with transcripts of pVDCCIII(A).

B. Isolation of DNA encoding the α_{1C} subunit

1. Reference List of Partial α_{1C} cDNA clones

Numerous α_{1C}-specific cDNA clones were isolated in order to characterize the α_{1C} coding sequence, the α_{1C} initiation of translation, and an alternatively spliced region of α_{1C}. SEQ ID No. 3 sets forth the characterized α_{1C} coding sequence (nt 1 to 5904) and deduced amino acid sequence. SEQ ID No. 4 and No. 5 encode two possible amino terminal ends of the α_{1C} protein. SEQ ID No. 6 encodes an alternative exon for the IV S3 transmembrane domain. Shown below is a list of clones used to characterize the α_{1C} sequence and the nucleotide position of each clone relative to the characterized α_{1C} sequence (SEQ ID No. 3). The isolation and characterization of these cDNA clones are described below (Example II.B.2).

IMR32	1.66	nt 1 to 916, SEQ ID No. 3
		nt 1 to 132, SEQ ID No. 4
IMR32	1.157	nt 1 to 873, SEQ ID No. 3
		nt 1 to 89, SEQ ID No. 5
IMR32	1.67	nt 50 to 1717, SEQ ID No. 3
*IMR32	1.86	nt 1366 to 2583, SEQ ID No. 3
@1.16G		nt 758 to 867, SEQ ID No. 3
IMR32	1.37	nt 2804 to 5904, SEQ ID No. 3
CNS	1.30	nt 2199 to 3903, SEQ ID No. 3
		nt 1 to 84 of alternative exon, SEQ ID No. 6
IMR32	1.38	nt 2448 to 4702, SEQ ID No. 3

nt 1 to 84 of alternative exon, SEQ ID No. 6

* IMR32 1.86 has a 73 nt deletion compared to the rabbit cardiac muscle calcium channel α_1 subunit cDNA sequence.

@1.16G is an α_{1C} genomic clone.

2. Isolation and characterization of clones described in Example II.B.1.

a. CNS 1.30

Approximately 1x 10^6 recombinants of the human thalamus cDNA library No. 6 (Example I.B.6.) were screened with fragments of the rabbit skeletal muscle calcium channel α_1 cDNA described in Example II.A.2.a. The hybridization was performed using standard hybridization conditions (Example I.C.) and the filters were washed under low stringency (Example I.C.). Six positive plaques were identified, one of which was CNS 1.30. CNS 1.30 was plaque purified, restriction mapped, subcloned, and characterized by DNA sequencing. CNS 1.30 encodes α_{1C}-specific sequence nt 2199 to 3903 (SEQ ID No. 3) followed by nt 1 to 84 of one of two identified alternative α_{1C} exons (SEQ ID No. 6). 3′ of SEQ ID No. 6, CNS 1.30 contains an intron and, thus, CNS 1.30 encodes a partially spliced α_{1C} transcript.

b. 1.16G

Approximately 1x 10^6 recombinants of a λEMBL3-based human genomic DNA library (Cat # HL1006d Clontech Corp., Palo Alto, CA) were screened using a rabbit skeletal muscle cDNA fragment (nt -78 to 1006, Example II.A.2.a.). The hybridization was performed using standard hybridization conditions (Example I.C.) and the filters were washed under low stringency (Example I.C.). Fourteen positive plaques were identified, one of which was 1.16G. Clone 1.16G was plaque purified, restriction mapped, subcloned, and portions were characterized by DNA sequencing. DNA

sequencing revealed that 1.16G encodes α_{1C}-specific sequence as described in Example II.B.1.

c. IMR32 1.66 and IMR32 1.67

Approximately 1x 10^6 recombinants of IMR32 cDNA library #5 (Example I.B.5.) were screened with a 151 bp *KpnI-SacI* fragment of 1.16G (Example II.B.2.b.) encoding α_{1C} sequence (nt 758 to 867, SEQ ID No. 3). The hybridization was performed using standard hybridization conditions (Example I.C.). The filters were then washed in 0.5 x SSPE at 65°C. Of the positive plaques, IMR32 1.66 and IMR32 1.67 were identified. The hybridizing plaques were purified, restriction mapped, subcloned, and characterized by DNA sequencing. Two of these cDNA clones, IMR32 1.66 and 1.67, encode α_{1C} subunits as described (Example II.B.1.). In addition, IMR32 1.66 encodes a partially spliced α_{1C} transcript marked by a GT splice donor dinucleotide beginning at the nucleotide 3′ of nt 916 (SEQ ID No. 3). The intron sequence within 1.66 is 101 nt long. IMR32 1.66 encodes the α_{1C} initiation of translation, nt 1 to 3 (SEQ ID No. 3) and 132 nt of 5′ untranslated sequence (SEQ ID No. 4) precede the start codon in IMR32 1.66.

d. IMR32 1.37 and IMR32 1.38

Approximately 2 x 10^6 recombinants of IMR32 cDNA library #1 (Example I.B.1.) were screened with the CNS 1.30 cDNA fragment (Example II.B.2.a.). The hybridization was performed using low stringency hybridization conditions (Example I.C.) and the filters were washed under low stringency (Example I.C.). Four positive plaques were identified, plaque purified, restriction mapped, subcloned, and characterized by DNA sequencing. Two of the clones, IMR32 1.37 and IMR32 1.38 encode α_{1C}-specific sequences as described in Example II.B.1.

DNA sequence comparison of IMR32 1.37 and IMR32 1.38 revealed that the α_{1C} transcript includes two exons that encode the IVS3 transmembrane domain. IMR32 1.37 has a

single exon, nt 3904 to 3987 (SEQ ID No. 3) and IMR32 1.38 appears to be anomalously spliced to contain both exons juxtaposed, nt 3904 to 3987 (SEQ ID No. 3) followed by nt 1 to 84 (SEQ ID No. 6). The alternative splice of the α_{1C} transcript to contain either of the two exons encoding the IVS3 region was confirmed by comparing the CNS 1.30 sequence to the IMR32 1.37 sequence. CNS 1.30 contains nt 1 to 84 (SEQ ID No. 6) preceded by the identical sequence contained in IMR32 1.37 for nt 2199 to 3903 (SEQ ID No. 3). As described in Example II.B.2.a., an intron follows nt 1 to 84 (SEQ ID No. 6). Two alternative exons have been spliced adjacent to nt 3903 (SEQ ID No. 3) represented by CNS 1.30 and IMR32 1.37.

e. IMR32 1.86

IMR32 cDNA library #1 (Example I.B.1.) was screened in duplicate using oligonucleotide probes 90-9 (nt 1462 to 1491, SEQ ID No. 3) and 90-12 (nt 2496 to 2520, SEQ ID No. 3). These oligonucleotide probes were chosen in order to isolate a clone that encodes the α_{1C} subunit between the 3′ end of IMR32 1.67 (nt 1717, SEQ ID No. 3) and the 5′ end of CNS 1.30 (nt 2199, SEQ ID No. 3). The hybridization conditions were standard hybridization conditions (Example I.C.) with the exception that the 50% deionized formamide was reduced to 20%. The filters were washed under low stringency (Example I.C.). Three positive plaques were identified one of which was IMR32 1.86. IMR32 1.86 was plaque purified, subcloned, and characterized by restriction mapping and DNA sequencing. IMR32 1.86 encodes α_{1C} sequences as described in Example II.B.1. Characterization by DNA sequencing revealed that IMR32 1.86 contains a 73 nt deletion compared to the DNA encoding rabbit cardiac muscle calcium channel α_1 subunit [Mikami *et al.* (1989) *Nature 340*:230], nt 2191 to 2263. These missing nucleotides correspond to nt 2176-2248 of SEQ ID No. 3. Because the 5′-end of CNS 1.30 overlaps the 3′-end of IMR32

1.86, some of these missing nucleotides, i.e., nt 2205-2248 of SEQ ID No. 3, are accounted for by CNS 1.30. The remaining missing nucleotides of the 73 nucleotide deletion in IMR32 1.86 (i.e., nt 2176-2204 SEQ ID No. 3) were determined by PCR analysis of dbcAMP-induced IMR32 cell RNA. The 73 nt deletion is a frame-shift mutation and, thus, needs to be corrected. The exact human sequence through this region, (which has been determined by the DNA sequence of CNS 1.30 and PCR analysis of IMR32 cell RNA) can be inserted into IMR32 1.86 by standard methods, *e.g.*, replacement of a restriction fragment or site-directed mutagenesis.

f. IMR32 1.157

One million recombinants of IMR32 cDNA library #4 (Example I.B.4.) were screened with an *Xho*I-*Eco*RI fragment of IMR32 1.67 encoding α_{1C} nt 50 to 774 (SEQ ID No. 3). The hybridization was performed using standard hybridization conditions (Example I.C.). The filters were washed under high stringency (Example I.C.). One of the positive plaques identified was IMR32 1.157. This plaque was purified, the insert was restriction mapped and subcloned to a standard plasmid vector pGEM7Z (Promega, Madison, WI). The DNA was characterized by sequencing. IMR32 1.157 appears to encodes an alternative 5′ portion of the α_{1C} sequence beginning with nt 1 to 89 (SEQ ID No. 5) and followed by nt 1 to 873 (SEQ ID No. 3). Analysis of the 1.66 and 1.157 5′ sequence is described below (Example II.B.3.).

3. Characterization of the α_{1C} initiation of translation site

Portions of the sequences of IMR32 1.157 (nt 57 to 89, SEQ ID No. 5; nt 1 to 67, SEQ ID No. 3), IMR32 1.66 (nt 100 to 132, SEQ ID No. 4; nt 1 to 67, SEQ ID No. 3), were compared to he rabbit lung CaCB-receptor cDNA sequence, nt -33 to 67 [Biel *et al.* (1990) *FEBS Lett. 269*:409]. The human sequences are possible alternative 5′ ends of the α_{1C}

transcript encoding the region of initiation of translation. IMR32 1.66 closely matches the CaCB receptor cDNA sequence and diverges from the CaCB receptor cDNA sequence in the 5′ direction beginning at nt 122 (SEQ ID No. 4). The start codon identified in the CaCB receptor cDNA sequence is the same start codon used to describe the α_{1C} coding sequence, nt 1 to 3 (SEQ ID No. 3). The functional significance of the IMR32 1.157 sequence, nt 1 to 89 (SEQ ID No. 5), is not clear. Chimeras containing sequence between 1.157 and the α_{1C} coding sequence can be constructed and functional differences can be tested.

C. Isolation of partial cDNA clones encoding the α_{1B} subunit and construction of a full-length clone

A human basal ganglia cDNA library was screened with the rabbit skeletal muscle α_1 subunit cDNA fragments (see Example II.A.2.a for description of fragments) under low stringency conditions. One of the hybridizing clones was used to screen an IMR32 cell cDNA library to obtain additional partial α_{1B} cDNA clones, which were in turn used to further screen an IMR32 cell cDNA library for additional partial cDNA clones. One of the partial IMR32 α_{1B} clones was used to screen a human hippocampus library to obtain a partial α_{1B} clone encoding the 3′ end of the α_{1B} coding sequence. The sequence of some of the regions of the partial cDNA clones was compared to the sequence of products of PCR analysis of IMR32 cell RNA to determine the accuracy of the cDNA sequences.

PCR analysis of IMR32 cell RNA and genomic DNA using oligonucleotide primers corresponding to sequences located 5′ and 3′ of the STOP codon of the DNA encoding the α_{1B} subunit revealed an alternatively spliced α_{1B}-encoding mRNA in IMR32 cells. This second mRNA product is the result of differential splicing of the α_{1B} subunit transcript to include another exon that is not present in the mRNA corresponding to the other 3′ α_{1B} cDNA sequence that was

initially isolated. To distinguish these splice variants of the α_{1B} subunit, the subunit encoded by a DNA sequence corresponding to the form containing the additional exon is referred to as α_{1B-1} (SEQ ID No. 7), whereas the subunit encoded by a DNA sequence corresponding to the form lacking the additional exon is referred to as α_{1B-2} (SEQ ID No. 8). The sequence of α_{1B-1} diverges from that of α_{1B-2} beginning at nt 6633 (SEQ ID No. 7). Following the sequence of the additional exon in α_{1B-1} (nt 6633-6819; SEQ ID No. 7), the α_{1B-1} and α_{1B-2} sequences are identical (i.e., nt 6820-7362 in SEQ ID No. 7 and nt 6633-7175 in SEQ ID No. 8). SEQ ID No. 7 and No. 8 set forth 143 nt of 5′ untranslated sequence (nt 1-143) as well as 202 nt of 3′ untranslated sequence (nt 7161-7362, SEQ ID No. 7) of the DNA encoding α_{1B-1} and 321 nt of 3′ untranslated sequence (nt 6855-7175, SEQ ID No. 8) of the DNA encoding α_{1B-2}.

PCR analysis of the IS6 region of the α_{1B} transcript revealed what appear to be additional splice variants based on multiple fragment sizes seen on an ethidium bromide-stained agarose gel containing the products of the PCR reaction.

A full-length α_{1B-1} cDNA clone designated pcDNA-α_{1B-1} was prepared in an eight-step process as follows.

STEP 1: The *Sac*I restriction site of pGEM3 (Promega, Madison, WI) was destroyed by digestion at the *Sac*I site, producing blunt ends by treatment with T4 DNA polymerase, and religation. The new vector was designated pGEMΔSac.

STEP 2: Fragment 1 (*Hind*III/*Kpn*I; nt 2337 to 4303 of SEQ ID No. 7) was ligated into *Hind*III/*Kpn*I digested pGEM3ΔSac to produce pα1.177HK.

STEP 3: Fragment 1 has a 2 nucleotide deletion (nt 3852 and 3853 of SEQ ID No. 7). The deletion was repaired by inserting a PCR fragment (fragment 2) of IMR32 RNA into pα1.177HK. Thus, fragment 2

(*Nar*I/*Kpn*I; nt 3828 to 4303 of SEQ ID No. 7) was inserted into *Nar*I/*Kpn*I digested pα1.177HK replacing the *Nar*I/*Kpn*I portion of fragment 1 and producing pα1.177HK/PCR.

STEP 4: Fragment 3 (*Kpn*I/*Kpn*I; nt 4303 to 5663 of SEQ ID No. 7) was ligated into *Kpn*I digested pα1.177HK/PCR to produce pα1B5'K.

STEP 5: Fragment 4 (*Eco*RI/*Hin*dIII; *Eco*RI adaptor plus nt 1 to 2337 of SEQ ID No. 7) and fragment 5 (*Hin*dIII/*Xho*I fragment of pα1B5'K; nt 2337 to 5446 of SEQ ID No. 7) were ligated together into *Eco*RI/*Xho*I digested pcDNA1 (Invitrogen, San Diego, CA) to produce pα1B5'.

STEP 6: Fragment 6 (*Eco*RI/*Eco*RI; *Eco*RI adapters on both ends plus nt 5749 to 7362 of SEQ ID No. 7) was ligated into *Eco*RI digested pBluescript II KS (Stratagene, La Jolla, CA) with the 5' end of the fragment proximal to the *Kpn*I site in the polylinker to produce pα1.230.

STEP 7: Fragment 7 (*Kpn*I/*Xho*I; nt 4303 to 5446 of SEQ ID No. 7), and fragment 8 (*Xho*I/*Csp*I; nt 5446 to 6259 of SEQ ID No. 7) were ligated into *Kpn*I/*Csp*I digested pα1.230 (removes nt 5749 to 6259 of SEQ ID No. 7 that was encoded in pα1.230 and maintains nt 6259 to 7362 of SEQ ID No. 7) to produce pα1B3'.

STEP 8: Fragment 9 (*Sph*I/*Xho*I; nt 4993 to 5446 of SEQ ID No. 7) and fragment 10 (*Xho*I/*Xba*I of pα1B3'; nt 5446 to 7319 of SEQ ID No. 7) were ligated into *Sph*I/*Xba*I digested pα1B5' (removes nt 4993 to 5446 of SEQ ID No. 7 that were encoded in pα1B5' and maintains nt 1 to 4850 of SEQ ID No. 7) to produce pcDNAα_{1B-1}.

The resulting construct, pcDNAα_{1B-1}, contains, in pCDNA1, a full-length coding region encoding α_{1B-1} (nt 144-

7362, SEQ ID No. 7), plus 5′ untranslated sequence (nt 1-143, SEQ ID No. 7) and 3′ untranslated sequence (nt 7161-7319, SEQ ID No. 7) under the transcriptional control of the CMV promoter.

EXAMPLE III: ISOLATION OF cDNA CLONES ENCODING THE HUMAN NEURONAL CALCIUM CHANNEL β_1 subunit

A. Isolation of partial cDNA clones encoding the β subunit and construction of a full-length clone encoding the β_1 subunit

A human hippocampus cDNA library was screened with the rabbit skeletal muscle calcium channel β_1 subunit cDNA fragment (nt 441 to 1379) [for isolation and sequence of the rabbit skeletal muscle calcium channel β_1 subunit cDNA, see U.S. Patent Application Serial NO. 482,384 or Ruth et *al*. (1989) *Science 245*:1115] using standard hybridization conditions (Example I.C.). A portion of one of the hybridizing clones was used to rescreen the hippocampus library to obtain additional cDNA clones. The cDNA inserts of hybridizing clones were characterized by restriction mapping and DNA sequencing and compared to the rabbit skeletal muscle calcium channel β_1 subunit cDNA sequence.

Portions of the partial β_1 subunit cDNA clones were ligated to generate a full-length clone encoding the entire β_1 subunit. SEQ ID No. 9 shows the β_1 subunit coding sequence (nt 1-1434) as well as a portion of the 3′ untranslated sequence (nt 1435-1546). The deduced amino acid sequence is also provided in SEQ ID No. 9. In order to perform expression experiments, full-length β_1 subunit cDNA clones were constructed as follows.

Step 1: DNA fragment 1 (~800 bp of 5′ untranslated sequence plus nt 1-277 of SEQ ID No. 9) was ligated to DNA fragment 2 (nt 277-1546 of SEQ ID No. 9 plus 448 bp of intron sequence) and cloned into pGEM7Z. The resulting plasmid, pβ1-1.18, contained a full-length β_1 subunit clone that included a 448-bp intron.

Step 2: To replace the 5′ untranslated sequence of pβ1-1.18 with a ribosome binding site, a double-stranded adapter was synthesized that contains an *Eco*RI site, sequence encoding a ribosome binding site (5′-ACCACC-3′) and nt 1-25 of SEQ ID No. 9. The adapter was ligated to *Sma*I-digested pβ1-1.18, and the products of the ligation reaction were digested with *Eco*RI.

Step 3: The *Eco*RI fragment from step 2 containing the *Eco*RI adapter, efficient ribosome binding site and nt 1-1546 of SEQ ID No. 9 plus intron sequence was cloned into a plasmid vector and designated pβ1-1.18RBS. The *Eco*RI fragment of pβ1-1.18RBS was subcloned into *Eco*RI-digested pcDNA1 with the initiation codon proximal to CMV promoter to form pHBCaCHβ_{1a}RBS(A).

Step 4: To generate a full-length clone encoding the β_1 subunit lacking intron sequence, DNA fragment 3 (nt 69-1146 of SEQ ID No. 9 plus 448 bp of intron sequence followed by nt 1147-1546 of SEQ ID No. 9), was subjected to site-directed mutagenesis to delete the intron sequence, thereby yielding pβ1(-). The *Eco*RI-*Xho*I fragment of pβ1-1.18RBS (containing of the ribosome binding site and nt 1-277 of SEQ ID No. 9) was ligated to the *Xho*I-*Eco*RI fragment of pβ1(-) (containing of nt 277-1546 of SEQ ID No. 9) and cloned into pcDNA1 with the initiation of translation proximal to the CMV promoter. The resulting expression plasmid was designated pHBCaCHβ_{1b}RBS(A).

B. Splice Variant β_{1-3}

DNA sequence analysis of the DNA clones encoding the β_1 subunit indicated that in the CNS at least two alternatively spliced forms of the same human β_1 subunit primary transcript are expressed. One form is represented by the sequence shown in SEQ ID No. 9 and is referred to as β_{1-2}. The sequences of β_{1-2} and the alternative form,

β_{1-3}, diverge at nt 1334 (SEQ ID No. 9). The complete β_{1-3} sequence (nt 1-1851), including 3′ untranslated sequence (nt 1795-1851), is set forth in SEQ ID No. 10.

EXAMPLE IV: ISOLATION OF cDNA CLONES ENCODING THE HUMAN NEURONAL CALCIUM CHANNEL α_2-subunit

A. Isolation of cDNA clones

The complete human neuronal α_2 coding sequence (nt 35-3307) plus a portion of the 5′ untranslated sequence (nt 1 to 34) as well as a portion of the 3′ untranslated sequence (nt 3308-3600) is set forth in SEQ ID No. 11.

To isolate DNA encoding the human neuronal α_2 subunit, human α_2 genomic clones first were isolated by probing human genomic Southern blots using a rabbit skeletal muscle calcium channel α_2 subunit cDNA fragment [nt 43 to 272, Ellis *et al.* (1988) *Science 240*:1661]. Human genomic DNA was digested with *Eco*RI, electrophoresed, blotted, and probed with the rabbit skeletal muscle probe using standard hybridization conditions (Example I.C.) and low stringency washing conditions (Example I.C.). Two restriction fragments were identified, 3.5 kb and 3.0 kb. These *Eco*RI restriction fragments were cloned by preparing a λgt11 library containing human genomic *Eco*RI fragments ranging from 2.2 kb to 4.3 kb. The library was screened as described above using the rabbit α_2 probe, hybridizing clones were isolated and characterized by DNA sequencing. HGCaCHα2.20 contained the 3.5 kb fragment and HGCaCHα2.9 contained the 3.0 kb fragment.

Restriction mapping and DNA sequencing revealed that HGCaCHα2.20 contains an 82 bp exon (nt 130 to 211 of the human α_2 coding sequence, SEQ ID No. 11) on a 650 bp *Pst*I-*Xba*I restriction fragment and that HGCaCHα2.9 contains 105 bp of an exon (nt 212 to 316 of the coding sequence, SEQ ID No. 11) on a 750 bp *Xba*I-*Bgl*II restriction fragment. These restriction fragments were used to screen the human basal ganglia cDNA library (Example II.C.2.a.). HBCaCHα2.1

was isolated (nt 29 to 1163, SEQ ID No. 11) and used to screen a human brain stem cDNA library (ATCC Accession No. 37432) obtained from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD. 20852. Two clones were isolated, HBCaCHα2.5 (nt 1 to 1162, SEQ ID No. 11) and HBCaCHα2.8 (nt 714 to 1562, SEQ ID No. 11, followed by 1600 nt of intervening sequence). A 2400 bp fragment of HBCaCHα2.8 (beginning at nt 759 of SEQ ID No. 11 and ending at a *Sma*I site in the intron) was used to rescreen the brain stem library and to isolate HBCaCHα2.11 (nt 879 to 3600, SEQ ID No. 11). Clones HBCaCHα2.5 and HBCaCHα2.11 overlap to encode an entire human brain α_2 protein.

B. Construction of pHBCaCHα_2A

To construct pHBCaCHα_2A containing DNA encoding a full-length human calcium channel α_2 subunit, an (*Eco*RI)-*Pvu*II fragment of HBCaCHα2.5 (nt 1 to 1061, SEQ ID No. 11, *Eco*RI adapter, *Pvu*II partial digest) and a *Pvu*II-*Pst*I fragment of HBCaCHα2.11 (nt 1061 to 2424 SEQ ID No. 11; *Pvu*II partial digest) were ligated into *Eco*RI-*Pst*I-digested pIBI24 (Stratagene, La Jolla, CA). Subsequently, an (*Eco*RI)-*Pst*I fragment (nt 1 to 2424 SEQ ID No. 11) was isolated and ligated to a *Pst*I-(*Eco*RI) fragment (nt 2424 to 3600 SEQ ID No. 11) of HBCaCHα2.11 in *Eco*RI-digested pIBI24 to produce DNA, HBCaCHα2, encoding a full-length human brain α_2 subunit. The 3600 bp *Eco*RI insert of HBCaCHα2 (nt 1 to 3600, SEQ ID No. 11) was subcloned into pcDNA1 (pHBCaCHα2A) with the methionine initiating codon proximal to the CMV promoter. The 3600 bp *Eco*RI insert of HBCaCHα2 was also subcloned into pSV2dHFR [Subramani *et al.* (1981). *Mol. Cell. Biol.* 1:854-864] which contains the SV40 early promoter, mouse dihydrofolate reductase (dhfr) gene, SV40 polyadenylation and splice sites and sequences required for maintenance of the vector in bacteria.

EXAMPLE V. DIFFERENTIAL PROCESSING OF THE HUMAN β_1 TRANSCRIPT AND THE HUMAN α_2 TRANSCRIPT

A. Differential processing of the β_1 transcript

PCR analysis of the human β_1 transcript present in skeletal muscle, aorta, hippocampus and basal ganglia, and HEK 293 cells revealed differential processing of the region corresponding to nt 615-781 of SEQ ID No. 9 in each of the tissues. Four different sequences that result in five different processed β_1 transcripts through this region were identified. The β_1 transcripts from the different tissues contained different combinations of the four sequences, except for one of the β_1 transcripts expressed in HEK 293 cells (β_{1-5}) which lacked all four sequences.

None of the β_1 transcripts contained each of the four sequences; however, for ease of reference, all four sequences are set forth end-to-end as a single long sequence in SEQ ID No. 12 . The four sequences that are differentially processed are sequence 1 (nt 14-34 in SEQ ID No. 12), sequence 2 (nt 35-55 in SEQ ID No. 12), sequence 3 (nt 56-190 in SEQ ID No. 12) and sequence 4 (nt 191-271 in SEQ ID No. 12). The forms of the β_1 transcript that have been identified include: (1) a form that lacks sequence 1 called β_{1-1} (expressed in skeletal muscle), (2) a form that lacks sequences 2 and 3 called β_{1-2} (expressed in CNS), (3) a form that lacks sequences 1, 2 and 3 called β_{1-4} (expressed in aorta and HEK cells) and (4) a form that lacks sequences 1-4 called β_{1-5} (expressed in HEK cells). Additionally, the β_{1-4} and β_{1-5} forms contain the guanine nucleotide (nt 13 in SEQ ID No. 12) which is absent in the β_{1-1} and β_{1-2} forms.

B. Differential processing of transcripts encoding the α_2 subunit.

The complete human neuronal α_2 coding sequence (nt 35-3307) plus a portion of the 5′ untranslated sequence (nt 1 to 34) as well as a portion of the 3′ untranslated sequence (nt 3308-3600) is set forth as SEQ ID No. 11.

PCR analysis of the human α_2 transcript present in skeletal muscle, aorta, and CNS revealed differential processing of the region corresponding to nt 1595-1942 of SEQ ID No. 11 in each of the tissues.

The analysis indicated that the primary transcript of the genomic DNA that includes the nucleotides corresponding to nt. 1595-1942 also includes an additional sequence (SEQ ID No. 13: 5'CCTATTGGTGTAGGTATACCAACAATTAATTT AAGAAAAAGGAGACCCAATATCCAG 3') inserted between nt. 1624 and 1625 of SEQ ID No. 11. Five alternatively spliced variant transcripts that differ in the presence or absence of one to three different portions of the region of the primary transcript that includes the region of nt. 1595-1942 of SEQ ID No. 11 plus SEQ ID No. 13 inserted between nt. 1624 and 1625 have been identified. The five α_2-encoding transcripts from the different tissues include different combinations of the three sequences, except for one of the α_2 transcripts expressed in aorta which lacks all three sequences. None of the α_2 transcripts contained each of the three sequences. The sequences of the three regions that are differentially processed are sequence 1 (SEQ ID No. 13), sequence 2 (5' AACCCCAAATCTCAG 3', which is nt. 1625-1639 of SEQ ID No. 11), and sequence 3 (5' CAAAAAAGGGCAAAATGAAGG 3', which is nt 1908-1928 of SEQ ID No. 11). The five α_2 forms identified are (1) a form that lacks sequence 3 called α_{2a} (expressed in skeletal muscle), (2) a form that lacks sequence 1 called α_{2b} (expressed in CNS), (3) a form that lacks sequences 1 and 2 called α_{2c} expressed in aorta), (4) a form that lacks sequences 1, 2 and 3 called α_{2d} (expressed in aorta) and (5) a form that lacks sequences 1 and 3 called α_{2e} (expressed in aorta).

EXAMPLE VI: ISOLATION OF DNA ENCODING A CALCIUM CHANNEL γ SUBUNIT FROM A HUMAN BRAIN cDNA LIBRARY

A. Isolation of DNA encoding the γ subunit

Approximately 1 x 10^6 recombinants from a λgt11-based human hippocampus cDNA library (Clontech catalog #HL1088b, Palo Alto, CA) were screened by hybridization to a 484 bp sequence of the rabbit skeletal muscle calcium channel γ subunit cDNA (nucleotides 621-626 of the coding sequence plus 438 nucleotides of 3′-untranslated sequence) contained in vector γJ10 [Jay, S. *et al.* (1990). *Science 248*:490-492]. Hybridization was performed using moderate stringency conditions (20% deionized formamide, 5x Denhardt's, 6 x SSPE, 0.2% SDS, 20 μg/ml herring sperm DNA, 42°C) and the filters were washed under low stringency (see Example I.C.). A plaque that hybridized to this probe was purified and insert DNA was subcloned into pGEM7Z. This cDNA insert was designated γ1.4.

B. Characterization of γ1.4

γ1.4 was confirmed by DNA hybridization and characterized by DNA sequencing. The 1500 bp *Sst*I fragment of γ1.4 hybridized to the rabbit skeletal muscle calcium channel γ subunit cDNA γJ10 on a Southern blot. SEQ analysis of this fragment revealed that it contains of approximately 500 nt of human DNA sequence and ~1000 nt of λgt11 sequence (included due to apparent destruction of one of the *Eco*RI cloning sites in λgt11). The human DNA sequence contains of 129 nt of coding sequence followed immediately by a translational STOP codon and 3′ untranslated sequence (SEQ ID No. 14).

To isolate the remaining 5′ sequence of the human γ subunit cDNA, human CNS cDNA libraries and/or preparations of mRNA from human CNS tissues can first be assayed by PCR methods using oligonucleotide primers based on the γ cDNA-specific sequence of γ1.4. Additional human neuronal γ subunit-encoding DNA can isolated from cDNA libraries that, based on the results of the PCR assay, contain γ-specific

amplifiable cDNA. Alternatively, cDNA libraries can be constructed from mRNA preparations that, based on the results of PCR assays, contain γ-specific amplifiable transcripts. Such libraries are constructed by standard methods using oligo dT to prime first-strand cDNA synthesis from poly A^+ RNA (see Example I.B.). Alternatively, first-strand cDNA can be specified by priming first-strand cDNA synthesis with a γ cDNA-specific oligonucleotide based on the human DNA sequence in γ1.4. A cDNA library can then be constructed based on this first-strand synthesis and screened with the γ-specific portion of γ1.4.

EXAMPLE VII: RECOMBINANT EXPRESSION OF HUMAN NEURONAL CALCIUM CHANNEL SUBUNIT-ENCODING cDNA AND RNA TRANSCRIPTS IN MAMMALIAN CELLS

A. Recombinant Expression of the Human Neuronal Calcium Channel α_2 subunit cDNA in DG44 Cells

1. Stable transfection of DG44 cells

DG44 cells [dhfr⁻ Chinese hamster ovary cells; see, *e.g.*, Urlaub, G. *et al.* (1986) *Som. Cell Molec. Genet. 12*:555-566] obtained from Lawrence Chasin at Columbia University were stably transfected by $CaPO_4$ precipitation methods [Wigler *et al.* (1979) *Proc. Natl. Acad. Sci. USA 76*:1373-1376] with pSV2dhfr vector containing the human neuronal calcium channel α_2-subunit cDNA (see Example IV) for polycistronic expression/selection in transfected cells. Transfectants were grown on 10% DMEM medium without hypoxanthine or thymidine in order to select cells that had incorporated the expression vector. Twelve transfectant cell lines were established as indicated by their ability to survive on this medium.

2. Analysis of α_2 subunit cDNA expression in transfected DG44 cells

Total RNA was extracted according to the method of Birnboim [(1988) *Nuc. Acids Res. 16*:1487-1497] from four of the DG44 cell lines that had been stably transfected with pSV2dhfr containing the human neuronal calcium channel α_2

subunit cDNA. RNA (~15 μg per lane) was separated on a 1% agarose formaldehyde gel, transferred to nitrocellulose and hybridized to the random-primed human neuronal calcium channel α_2 cDNA (hybridization: 50% formamide, 5 x SSPE, 5 x Denhardt's, 42° C.; wash :0.2 x SSPE, 0.1% SDS, 65° C.). Northern blot analysis of total RNA from four of the DG44 cell lines that had been stably transfected with pSV2dhfr containing the human neuronal calcium channel α_2 subunit cDNA revealed that one of the four cell lines contained hybridizing mRNA the size expected for the transcript of the α_2 subunit cDNA (5000 nt based on the size of the cDNA) when grown in the presence of 10 mM sodium butyrate for two days. Butyrate nonspecifically induces transcription and is often used for inducing the SV40 early promoter [Gorman, C. and Howard, B. (1983) *Nucleic Acids Res. 11*:1631]. This cell line, 44α_2-9, also produced mRNA species smaller (several species) and larger (6800 nt) than the size expected for the transcript of the α_2 cDNA (5000 nt) that hybridized to the α_2 cDNA-based probe. The 5000- and 6800-nt transcripts produced by this transfectant should contain the entire α_2 subunit coding sequence and therefore should yield a full-length α_2 subunit protein. A weakly hybridizing 8000-nucleotide transcript was present in untransfected and transfected DG44 cells. Apparently, DG44 cells transcribe a calcium channel α_2 subunit or similar gene at low levels. The level of expression of this endogenous α_2 subunit transcript did not appear to be affected by exposing the cells to butyrate before isolation of RNA for northern analysis.

Total protein was extracted from three of the DG44 cell lines that had been stably transfected with pSV2dhfr containing the human neuronal calcium channel α_2 subunit cDNA. Approximately 10^7 cells were sonicated in 300 μl of a solution containing 50 mM HEPES, 1 mM EDTA, 1 mM PMSF. An equal volume of 2x loading dye [Laemmli, U.K. (1970).

Nature 227:680] was added to the samples and the protein was subjected to electrophoresis on an 8% polyacrylamide gel and then electrotransferred to nitrocellulose. The nitrocellulose was incubated with polyclonal guinea pig antisera (1:200 dilution) directed against the rabbit skeletal muscle calcium channel α_2 subunit (obtained from K. Campbell, University of Iowa) followed by incubation with [^{125}I]-protein A. The blot was exposed to X-ray film at -70° C. Reduced samples of protein from the transfected cells as well as from untransfected DG44 cells contained immunoreactive protein of the size expected for the α_2 subunit of the human neuronal calcium channel (130-150 kDa). The level of this immunoreactive protein was higher in 44α_2-9 cells that had been grown in the presence of 10 mM sodium butyrate than in 44α_2-9 cells that were grown in the absence of sodium butyrate. These data correlate well with those obtained in northern analyses of total RNA from 44α_2-9 and untransfected DG44 cells. Cell line 44α_2-9 also produced a 110 kD immunoreactive protein that may be either a product of proteolytic degradation of the full-length α_2 subunit or a product of translation of one of the shorter (<5000 nt) mRNAs produced in this cell line that hybridized to the α_2 subunit cDNA probe.

B. Expression of DNA encoding human neuronal calcium channel α_1, α_2 and β_1 subunits in HEK cells

Human embryonic kidney cells (HEK 293 cells) were transiently and stably transfected with human neuronal DNA encoding calcium channel subunits. Individual transfectants were analyzed electrophysiologically for the presence of voltage-activated barium currents and functional recombinant voltage-dependent calcium channels were.

1. Transfection of HEK 293 cells

Separate expression vectors containing DNA encoding human neuronal calcium channel α_{1D}, α_2 and β_1 subunits,

plasmids pVDCCIII(A), pHBCaCHα_2A, and pHBCaCHβ_{1a}RBS(A), respectively, were constructed as described in Examples II.A.3, IV.B. and III.B.3., respectively. These three vectors were used to transiently co-transfect HEK 293 cells. For stable transfection of HEK 293 cells, vector pHBCaCHβ_{1b}RBS(A) (Example III.B.3.) was used in place of pHBCaCHβ_{1a}RBS(A) to introduce the DNA encoding the β_1 subunit into the cells along with pVDCCIII(A) and pHBCaCHα_2A.

a. Transient transfection

Expression vectors pVDCCIII(A), pHBCaCHα_2A and pHBCaCHβ_{1a}RBS(A) were used in two sets of transient transfections of HEK 293 cells (ATCC Accession No. CRL1573). In one transfection procedure, HEK 293 cells were transiently cotransfected with the α_1 subunit cDNA expression plasmid, the α_2 subunit cDNA expression plasmid, the β_1 subunit cDNA expression plasmid and plasmid pCMVβgal (Clontech Laboratories, Palo Alto, CA). Plasmid pCMVβgal contains the *lacZ* gene (encoding *E. coli* β-galactosidase) fused to the cytomegalovirus (CMV) promoter and was included in this transfection as a marker gene for monitoring the efficiency of transfection. In the other transfection procedure, HEK 293 cells were transiently co-transfected with the α_1 subunit cDNA expression plasmid pVDCCIII(A) and pCMVβgal. In both transfections, 2-4 x 10^6 HEK 293 cells in a 10-cm tissue culture plate were transiently co-transfected with 5 μg of each of the plasmids included in the experiment according to standard $CaPO_4$ precipitation transfection procedures (Wigler *et al.* (1979) *Proc. Natl. Acad. Sci. USA* 76:1373-1376). The transfectants were analyzed for β-galactosidase expression by direct staining of the product of a reaction involving β-galactosidase and the X-gal substrate [Jones, J.R. (1986) *EMBO* 5:3133-3142] and by measurement of β-galactosidase activity [Miller, J.H. (1972) Experiments in Molecular Genetics, pp. 352-355, Cold Spring Harbor Press]. To

evaluate subunit cDNA expression in these transfectants, the cells were analyzed for subunit transcript production (northern analysis), subunit protein production (immunoblot analysis of cell lysates) and functional calcium channel expression (electrophysiological analysis).

b. Stable transfection

HEK 293 cells were transfected using the calcium phosphate transfection procedure [*Current Protocols in Molecular Biology*, Vol. 1, Wiley Inter-Science, Supplement 14, Unit 9.1.1-9.1.9 (1990)]. Ten-cm plates, each containing one-to-two million HEK 293 cells, were transfected with 1 ml of DNA/calcium phosphate precipitate containing 5 μg pVDCCIII(A), 5 μg pHBCaCHα_2A, 5μg pHBCaCHβ_{1b}RBS(A), 5 μg pCMVBgal and 1 μg pSV2neo (as a selectable marker). After 10-20 days of growth in media containing 500 μg G418, colonies had formed and were isolated using cloning cylinders.

2. Analysis of HEK 293 cells transiently transfected with DNA encoding human neuronal calcium channel subunits

a. Analysis of β-galactosidase expression

Transient transfectants were assayed for β-galactosidase expression by β-galactosidase activity assays (Miller, J.H., (1972) Experiments in Molecular Genetics, pp. 352-355, Cold Spring Harbor Press) of cell lysates (prepared as described in Example VII.A.2) and staining of fixed cells (Jones, J.R. (1986) *EMBO 5*:3133-3142). The results of these assays indicated that approximately 30% of the HEK 293 cells had been transfected.

b. Northern analysis

PolyA+ RNA was isolated using the Invitrogen Fast Trak Kit (InVitrogen, San Diego, CA) from HEK 293 cells transiently transfected with DNA encoding each of the α_1, α_2 and β_1 subunits and the *lacZ* gene or the α_1 subunit and the *lacZ* gene. The RNA was subjected to electrophoresis on an agarose gel and transferred to nitrocellulose. The

nitrocellulose was then hybridized with one or more of the following radiolabeled probes: the *lacZ* gene, human neuronal calcium channel α_{1D} subunit-encoding cDNA, human neuronal calcium channel α_2 subunit-encoding cDNA or human neuronal calcium channel β_1 subunit-encoding cDNA. Two transcripts that hybridized with the α_1 subunit-encoding cDNA were detected in HEK 293 cells transfected with the DNA encoding the α_1, α_2, and β_1 subunits and the *lacZ* gene as well as in HEK 293 cells transfected with the α_1 subunit cDNA and the *lacZ* gene. One mRNA species was the size expected for the transcript of the α_1 subunit cDNA (8000 nucleotides). The second RNA species was smaller (4000 nucleotides) than the size expected for this transcript. RNA of the size expected for the transcript of the *lacZ* gene was detected in cells transfected with the α_1, α_2 and β_1 subunit-encoding cDNA and the *lacZ* gene and in cells transfected with the α_1 subunit cDNA and the *lacZ* gene by hybridization to the *lacZ* gene sequence.

RNA from cells transfected with the α_1, α_2 and β_1 subunit-encoding cDNA and the *lacZ* gene was also hybridized with the α_2 and β_1 subunit cDNA probes. Two mRNA species hybridized to the α_2 subunit cDNA probe. One species was the size expected for the transcript of the α_2 subunit cDNA (4000 nucleotides). The other species was larger (6000 nucleotides) than the expected size of this transcript. Multiple RNA species in the cells co-transfected with α_1, α_2 and β_1 subunit-encoding cDNA and the *lacZ* gene hybridized to the β_1 subunit cDNA probe. Multiple β-subunit transcripts of varying sizes were produced since the β subunit cDNA expression vector contains two potential polyA$^+$ addition sites.

c. Electrophysiological analysis

Individual transiently transfected HEK 293 cells were assayed for the presence of voltage-dependent barium currents using the whole-cell variant of the patch clamp

technique [Hamill et al. (1981). *Pflugers Arch. 391*:85-100]. HEK 293 cells transiently transfected with pCMVβgal only were assayed for barium currents as a negative control in these experiments. The cells were placed in a bathing solution that contained barium ions to serve as the current carrier. Choline chloride, instead of NaCl or KCl, was used as the major salt component of the bath solution to eliminate currents through sodium and potassium channels. The bathing solution contained 1 mM $MgCl_2$ and was buffered at pH 7.3 with 10 mM HEPES (pH adjusted with sodium or tetraethylammonium hydroxide). Patch pipettes were filled with a solution containing 135 mM CsCl, 1 mM $MgCl_2$, 10 mM glucose, 10 mM EGTA, 4 mM ATP and 10 mM HEPES (pH adjusted to 7.3 with tetraethylammonium hydroxide). Cesium and tetraethylammonium ions block most types of potassium channels. Pipettes were coated with Sylgard (Dow-Corning, Midland, MI) and had resistances of 1-4 megohm. Currents were measured through a 500 megohm headstage resistor with the Axopatch IC (Axon Instruments, Foster City, CA) amplifier, interfaced with a Labmaster (Scientific Solutions, Solon, OH) data acquisition board in an IBM-compatible PC. PClamp (Axon Instruments) was used to generate voltage commands and acquire data. Data were analyzed with pClamp or Quattro Professional (Borland International, Scotts Valley, CA) programs.

To apply drugs, "puffer" pipettes positioned within several micrometers of the cell under study were used to apply solutions by pressure application. The drugs used for pharmacological characterization were dissolved in a solution identical to the bathing solution. Samples of a 10 mM stock solution of Bay K 8644 (RBI, Natick, MA), which was prepared in DMSO, were diluted to a final concentration of 1 μM in 15 mM Ba^{2+}-containing bath solution before they were applied.

Twenty-one negative control HEK 293 cells (transiently transfected with the *lacZ* gene expression vector pCMVβgal only) were analyzed by the whole-cell variant of the patch clamp method for recording currents. Only one cell displayed a discernable inward barium current; this current was not affected by the presence of 1 μM Bay K 8644. In addition, application of Bay K 8644 to four cells that did not display Ba^{2+} currents did not result in the appearance of any currents.

Two days after transient transfection of HEK 293 cells with α_1, α_2 and β_1 subunit-encoding cDNA and the *lacZ* gene, individual transfectants were assayed for voltage-dependent barium currents. The currents in nine transfectants were recorded. Because the efficiency of transfection of one cell can vary from the efficiency of transfection of another cell, the degree of expression of heterologous proteins in individual transfectants varies and some cells do not incorporate or express the foreign DNA. Inward barium currents were detected in two of these nine transfectants. In these assays, the holding potential of the membrane was -90 mV. The membrane was depolarized in a series of voltage steps to different test potentials and the current in the presence and absence of 1 μM Bay K 8644 was recorded. The inward barium current was significantly enhanced in magnitude by the addition of Bay K 8644. The largest inward barium current (-160 pA) was recorded when the membrane was depolarized to 0 mV in the presence of 1 μM Bay K 8644. A comparison of the I-V curves, generated by plotting the largest current recorded after each depolarization versus the depolarization voltage, corresponding to recordings conducted in the absence and presence of Bay K 8644 illustrated the enhancement of the voltage-activated current in the presence of Bay K 8644.

Pronounced tail currents were detected in the tracings of currents generated in the presence of Bay K 8644 in HEK

293 cells transfected with α_1, α_2 and β_1 subunit-encoding cDNA and the *lacZ* gene, indicating that the recombinant calcium channels responsible for the voltage-activated barium currents recorded in this transfected appear to be DHP-sensitive.

The second of the two transfected cells that displayed inward barium currents expressed a -50 pA current when the membrane was depolarized from -90 mV. This current was nearly completely blocked by 200 μM cadmium, an established calcium channel blocker.

Ten cells that were transiently transfected with the DNA encoding the α_1 subunit and the *lacZ* gene were analyzed by whole-cell patch clamp methods two days after transfection. One of these cells displayed a 30 pA inward barium current. This current amplified 2-fold in the presence of 1 μM Bay K 8644. Furthermore, small tail currents were detected in the presence of Bay K 8644. These data indicate that expression of the human neuronal calcium channel α_{1D} subunit-encoding cDNA in HEK 293 yields a functional DHP-sensitive calcium channel.

3. Analysis of HEK 293 cells stably transfected with DNA encoding human neuronal calcium channel subunits

Individual stably transfected HEK 293 cells were assayed electrophysiologically for the presence of voltage-dependent barium currents as described for electrophysiological analysis of transiently transfected HEK 293 cells (see Example VII.B.2.c). In an effort to maximize calcium channel activity via cyclic-AMP-dependent kinase-mediated phosphorylation [Pelzer, et al. (1990) *Rev. Physiol. Biochem. Pharmacol. 114*:107-207], cAMP (Na salt, 250 μM) was added to the pipet solution and forskolin (10 μM) was added to the bath solution in some of the recordings. Qualitatively similar results were obtained whether these compounds were present or not.

Barium currents were recorded from stably transfected cells in the absence and presence of Bay K 8644 (1 μM). When the cell was depolarized to -10 mV from a holding potential of -90 mV in the absence of Bay K 8644, a current of approximately 35pA with a rapidly deactivating tail current was recorded. During application of Bay K 8644, an identical depolarizing protocol elicited a current of approximately 75 pA, accompanied by an augmented and prolonged tail current. The peak magnitude of currents recorded from this same cell as a function of a series of depolarizing voltages were assessed. The responses in the presence of Bay K 8644 not only increased, but the entire current-voltage relation shifted about -10 mV. Thus, three typical hallmarks of Bay K 8644 action, namely increased current magnitude, prolonged tail currents, and negatively shifted activation voltage, were observed, clearly indicating the expression of a DHP-sensitive calcium channel in these stably transfected cells. No such effects of Bay K 8644 were observed in untransfected HEK 293 cells, either with or without cAMP or forskolin.

C. Use of pCMV-based vectors and pcDNA1-based vectors for expression of DNA encoding human neuronal calcium channel subunits

1. Preparation of constructs

To determine if the levels of recombinant expression of human calcium channel subunit-encoding DNA in host cells could be enhanced by using pCMV-based instead of pcDNA1-based expression vectors, additional expression vectors were constructed. The full-length α_{1D} cDNA from pVDCCIII(A) (see Example II.A.3.d), the full-length α_2 cDNA, contained on a 3600 bp *Eco*RI fragment from HBCaCHα_2 (see Example IV.B) and a full-length β_1 subunit cDNA from pHBCaCHβ_{1b}RBS(A) (see Example III.B.3) were separately subcloned into plasmid pCMVβgal. Plasmid pCMVβgal was digested with *Not*I to remove the *lacZ* gene. The remaining vector portion of the plasmid, referred to as pCMV, was blunt-ended at the *Not*I

sites. The full-length α_2-encoding DNA and β_1-encoding DNA, contained on separate *Eco*RI fragments, were isolated, blunt-ended and separately ligated to the blunt-ended vector fragment of pCMV locating the cDNAs between the CMV promoter and SV40 polyadenylation sites in pCMV. To ligate the α_{1D}-encoding cDNA with pCMV, the restriction sites in the polylinkers immediately 5′ of the CMV promoter and immediately 3′ of the SV40 polyadenylation site were removed from pCMV. A polylinker was added at the *Not*I site. The polylinker had the following sequence of restriction enzyme recognition sites:

GGCCGC CG	*Eco*RI site	*Sal*I site	*Pst*I site	*Eco*RV site	*Hin*dIII site	*Xba*II site	GT CACCGG

*Not*I — Destroys *Not*

The α_{1D}-encoding DNA, isolated as a *Bam*HI/*Xho*I fragment from pVDCCIII(A), was then ligated to *Xba*II/*Sal*I-digested pCMV to place it between the CMV promoter and SV40 polyadenylation site.

Plasmid pCMV contains the CMV promoter as does pcDNA1, but differs from pcDNA1 in the location of splice donor/splice acceptor sites relative to the inserted subunit-encoding DNA. After inserting the subunit-encoding DNA into pCMV, the splice donor/splice acceptor sites are located 3′ of the CMV promoter and 5′ of the subunit-encoding DNA start codon. After inserting the subunit-encoding DNA into pcDNA1, the splice donor/splice acceptor sites are located 3′ of the subunit cDNA stop codon.

2. Transfection of HEK 293 cells

HEK 293 cells were transiently co-transfected with the α_{1D}, α_2 and β_1 subunit-encoding DNA in pCMV or with the α_{1D}, α_2 and β subunit-encoding DNA in pcDNA1 (vectors pVDCCIII(A), pHBCaCHα_2A and pHBCaCHβ_{1b}RBS(A), respectively), as described in Example VII.B.1.a. Plasmid pCMVβgal was included in each transfection to as a measure of

transfection efficiency. The results of β-galactosidase assays of the transfectants (see Example VII.B.2.), indicated that HEK 293 cells were transfected equally efficiently with pCMV- and pcDNA1-based plasmids.

3. Northern analysis

Total and polyA$^+$ RNA were isolated from the transiently transfected cells as described in Examples VII.A.2 and VII.B.2.b. Northern blots of the RNA were hybridized with the following radiolabeled probes: α_{1D} cDNA, human neuronal calcium channel α_2 subunit cDNA and DNA encoding the human neuronal calcium channel β_1 subunit. Messenger RNA of sizes expected for α_{1D}, α_2 and β_1 subunit transcripts were detected in all transfectants. A greater amount of the α_{1D} transcript was present in cells that were co-transfected with pCMV-based plasmids then in cells that were co-transfected with pcDNA1-based plasmids. Equivalent amounts of α_2 and β_1 subunit transcripts were detected in all transfectants.

D. Expression in Xenopus laevis oöcytes of RNA encoding human neuronal calcium channel subunits

Various combinations of the transcripts of DNA encoding the human neuronal α_{1D}, α_2 and β_1 subunits prepared *in vitro* were injected into *Xenopus laevis* oöcytes. Those injected with combinations that included a_{1D} exhibited voltage-activated barium currents.

1. Preparation of transcripts

Transcripts encoding the human neuronal calcium channel α_{1D}, α_2 and β_1 subunits were synthesized according to the instructions of the mCAP mRNA CAPPING KIT (Strategene, La Jolla, CA catalog #200350). Plasmids pVDCC III.RBS(A), containing of pcDNA1 and the α_{1D} cDNA that begins with a ribosome binding site and the eighth ATG codon of the coding sequence (see Example III.A.3.d), plasmid pHBCaCHα_1A containing of pcDNA1 and an α_2 subunit cDNA (see Example IV), and plasmid pHBCaCHβ_{1b}RBS(A) containing pcDNA1 and the

β_1 DNA lacking intron sequence and containing a ribosome binding site (see Example III), were linearized by restriction digestion. The α_{1D} cDNA- and α_2 subunit-encoding plasmids were digested with *Xho*I, and the β_1 subunit-encoding plasmid was digested with *Eco*RV. The DNA insert was transcribed with T7 RNA polymerase.

2. Injection of oöcytes

Xenopus laevis oöcytes were isolated and defolliculated by collagenase treatment and maintained in 100 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.6, 20 μg/ml ampicillin and 25 μg/ml streptomycin at 19-25°C for 2 to 5 days after injection and prior to recording. For each transcript that was injected into the oöcyte, 6 ng of the specific mRNA was injected per cell in a total volume of 50 nl.

3. Intracellular voltage recordings

Injected oöcytes were examined for voltage-dependent barium currents using two-electrode voltage clamp methods [Dascal, N. (1987) *CRC Crit. Rev. Biochem. 22*:317]. The pClamp (Axon Instruments) software package was used in conjunction with a Labmaster 125 kHz data acquisition interface to generate voltage commands and to acquire and analyze data. Quattro Professional was also used in this analysis. Current signals were digitized at 1-5 kHz, and filtered appropriately. The bath solution contained of the following: 40 mM $BaCl_2$, 36 mM tetraethylammonium chloride (TEA-Cl), 2 mM KCl, 5 mM 4-aminopyridine, 0.15 mM niflumic acid, 5 mM HEPES, pH 7.6.

a. Electrophysiological analysis of oöcytes injected with transcripts encoding the human neuronal calcium channel α_1, α_2 and β_1-subunits

Uninjected oöcytes were examined by two-electrode voltage clamp methods and a very small (25 nA) endogenous inward Ba^{2+} current was detected in only one of seven analyzed cells.

Oöcytes coinjected with α_{1D}, α_2 and β_1 subunit transcripts expressed sustained inward barium currents upon depolarization of the membrane from a holding potential of -90 mV or -50 mV (154 ± 129 nA, n=21). These currents typically showed little inactivation when test pulses ranging from 140 to 700 msec. were administered. Depolarization to a series of voltages revealed currents that first appeared at approximately -30 mV and peaked at approximately 0 mV.

Application of the DHP Bay K 8644 increased the magnitude of the currents, prolonged the tail currents present upon repolarization of the cell and induced a hyperpolarizing shift in current activation. Bay K 8644 was prepared fresh from a stock solution in DMSO and introduced as a 10x concentrate directly into the 60 μl bath while the perfusion pump was turned off. The DMSO concentration of the final diluted drug solutions in contact with the cell never exceeded 0.1%. Control experiments showed that 0.1% DMSO had no effect on membrane currents.

Application of the DHP antagonist nifedipine (stock solution prepared in DMSO and applied to the cell as described for application of Bay K 8644) blocked a substantial fraction (91 ± 6%, n=7) of the inward barium current in oöcytes coinjected with transcripts of the α_{1D}, α_2 and β_1 subunits. A residual inactivating component of the inward barium current typically remained after nifedipine application. The inward barium current was blocked completely by 50 μM Cd^{2+}, but only approximately 15% by 100 μM Ni^{2+}.

The effect of ωCgTX on the inward barium currents in oöcytes co-injected with transcripts of the α_{1D}, α_2, and β_1 subunits was investigated. ωCgTX (Bachem, Inc., Torrance CA) was prepared in the 15 mM $BaCl_2$ bath solution plus 0.1% cytochrome C (Sigma) to serve as a carrier protein.

Control experiments showed that cytochrome C had no effect on currents. A series of voltage pulses from a -90 mV holding potential to 0 mV were recorded at 20 msec. intervals. To reduce the inhibition of ωCgTX binding by divalent cations, recordings were made in 15 mM $BaCl_2$, 73.5 mM tetraethylammonium chloride, and the remaining ingredients identical to the 40 mM Ba^{2+} recording solution. Bay K 8644 was applied to the cell prior to addition to ωCgTX in order to determine the effect of ωCgTX on the DHP-sensitive current component that was distinguished by the prolonged tail currents. The inward barium current was blocked weakly (54 ± 29%, n=7) and reversibly by relatively high concentrations (10-15 μM) of ωCgTX. The test currents and the accompanying tail currents were blocked progressively within two to three minutes after application of ωCgTX, but both recovered partially as the ωCgTX was flushed from the bath.

b. Analysis of oöcytes injected with only a transcripts encoding the human neuronal calcium channel α_{1D} or transcripts encoding an α_{1D} and other subunits

The contribution of the α_2 and β_1 subunits to the inward barium current in oöcytes injected with transcripts encoding the α_{1D}, α_2 and β_1 subunits was assessed by expression of the α_{1D} subunit alone or in combination with either the β_1 subunit or the α_2 subunit. In oöcytes injected with only the transcript of a α_{1D} cDNA, no Ba^{2+} currents were detected (n=3). In oöcytes injected with transcripts of α_{1D} and β_1 cDNAs, small (108 ± 39 nA) Ba^{2+} currents were detected upon depolarization of the membrane from a holding potential of -90 mV that resembled the currents observed in cells injected with transcripts of α_{1D}, α_2 and β_1 cDNAs, although the magnitude of the current was less. In two of the four oöcytes injected with transcripts of the α_{1D}-encoding and β_1-encoding DNA, the Ba^{2+} currents

exhibited a sensitivity to Bay K 8644 that was similar to the Bay K 8644 sensitivity of Ba^{2+} currents expressed in oöcytes injected with transcripts encoding the α_{1D} α_1-, α_2- and β_1 subunits.

Three of five oöcytes injected with transcripts encoding the α_{1D} and α_2 subunits exhibited very small Ba^{2+} currents (15-30 nA) upon depolarization of the membrane from a holding potential of -90 mV. These barium currents showed little or no response to Bay K 8644.

c. Analysis of oöcytes injected with transcripts encoding the human neuronal calcium channel α_2 and/or β_1 subunit

To evaluate the contribution of the α_{1D} α_1-subunit to the inward barium currents detected in oöcytes co-injected with transcripts encoding the α_{1D}, α_2 and β_1 subunits, oöcytes injected with transcripts encoding the human neuronal calcium channel α_2 and/or β_1 subunits were assayed for barium currents. Oöcytes injected with transcripts encoding the α_2 subunit displayed no detectable inward barium currents (n=5). Oöcytes injected with transcripts encoding a β_1 subunit displayed measurable (54 ± 23 nA, n=5) inward barium currents upon depolarization and oöcytes injected with transcripts encoding the α_2 and β_1 subunits displayed inward barium currents that were approximately 50% larger (80 ± 61 nA, n=18) than those detected in oöcytes injected with transcripts of the β_1-encoding DNA only.

The inward barium currents in oöcytes injected with transcripts encoding the β_1 subunit or α_2 and β_1 subunits typically were first observed when the membrane was depolarized to -30 mV from a holding potential of -90 mV and peaked when the membrane was depolarized to 10 to 20 mV. Macroscopically, the currents in oöcytes injected with transcripts encoding the α_2 and β_1 subunits or with transcripts encoding the β_1 subunit were indistinguishable. In contrast to the currents in oöcytes co-injected with

transcripts of α_{1D}, α_2 and β_1 subunit cDNAs, these currents showed a significant inactivation during the test pulse and a strong sensitivity to the holding potential. The inward barium currents in oöcytes co-injected with transcripts encoding the α_2 and β_1 subunits usually inactivated to 10-60% of the peak magnitude during a 140-msec pulse and were significantly more sensitive to holding potential than those in oöcytes co-injected with transcripts encoding the α_{1D}, α_2 and β_1 subunits. Changing the holding potential of the membranes of oöcytes co-injected with transcripts encoding the α_2 and β_1 subunits from -90 to -50 mV resulted in an approximately 81% (n=11) reduction in the magnitude of the inward barium current of these cells. In contrast, the inward barium current measured in oöcytes co-injected with transcripts encoding the α_{1D}, α_2 and β_1 subunits were reduced approximately 24% (n=11) when the holding potential was changed from -90 to -50 mV.

The inward barium currents detected in oöcytes injected with transcripts encoding the α_2 and β_1 subunits were pharmacologically distinct from those observed in oöcytes co-injected with transcripts encoding the α_{1D}, α_2 and β_1 subunits. Oöcytes injected with transcripts encoding the α_2 and β_1 subunits displayed inward barium currents that were insensitive to Bay K 8644 (n=11). Nifedipine sensitivity was difficult to measure because of the holding potential sensitivity of nifedipine and the current observed in oöcytes injected with transcripts encoding the α_2 and β_1 subunits. Nevertheless, two oöcytes that were co-injected with transcripts encoding the α_2 and β_1 subunits displayed measurable (25 to 45 nA) inward barium currents when depolarized from a holding potential of -50 mV. These currents were insensitive to nifedipine (5 to 10 μM). The inward barium currents in oöcytes injected with transcripts encoding the α_2 and β_1 subunits showed the same sensitivity to heavy metals as the currents detected in oöcytes

injected with transcripts encoding the α_{1D}, α_2 and β_1 subunits.

The inward barium current detected in oöcytes injected with transcripts encoding the human neuronal α_2 and β_1 subunits has pharmacological and biophysical properties that resemble calcium currents in uninjected *Xenopus* oöcytes. Because the amino acids of this human neuronal calcium channel β_1 subunit lack hydrophobic segments capable of forming transmembrane domains, it is unlikely that recombinant β_1 subunits alone can form an ion channel. It is more probable that a homologous endogenous α_1 subunit exists in oöcytes and that the activity mediated by such an α_1 subunit is enhanced by expression of a human neuronal β_1 subunit.

While the invention has been described with some specificity, modifications apparent to those with ordinary skill in the art may be made without departing from the scope of the invention. Since such modifications will be apparent to those of skill in the art, it is intended that this invention be limited only by the scope of the appended claims.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: HUMAN CALCIUM CHANNEL COMPOSITIONS AND METHODS

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7635 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 511..6996

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..510

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 6994..7635

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GGGCGAGCGC CTCCGTCCCC GGATGTGAGC TCCGGCTGCC CGCGGTCCCG AGCCAGCGGC      60

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GGGGAGCGCC GAGCGGCCCC GGCGGCCGGG CCGGCATCAC CGCGGCGTCT CTCCGCTAGA     180

GGAGGGGACA AGCCAGTTCT CCTTTGCAGC AAAAAATTAC ATGTATATAT TATTAAGATA     240

ATATATACAT TGGATTTTAT TTTTTTAAAA AGTTTATTTT GCTCCATTTT TGAAAAAGAG     300

AGAGCTTGGG TGGCGAGCGG TTTTTTTTTA AAATCAATTA TCCTTATTTT CTGTTATTTG     360

TCCCCGTCCC TCCCCACCCC CCTGCTGAAG CGAGAATAAG GGCAGGGACC GCGGCTCCTA     420

CCTCTTGGTG ATCCCCTTCC CCATTCCGCC CCCGCCCCAA CGCCCAGCAC AGTGCCCTGC     480

ACACAGTAGT CGCTCAATAA ATGTTCGTGG ATG ATG ATG ATG ATG ATG ATG AAA      534
                                    Met Met Met Met Met Met Met Lys
                                     1               5

AAA ATG CAG CAT CAA CGG CAG CAG CAA GCG GAC CAC GCG AAC GAG GCA      582
Lys Met Gln His Gln Arg Gln Gln Gln Ala Asp His Ala Asn Glu Ala
 10                  15                  20

AAC TAT GCA AGA GGC ACC AGA CTT CCT CTT TCT GGT GAA GGA CCA ACT      630
Asn Tyr Ala Arg Gly Thr Arg Leu Pro Leu Ser Gly Glu Gly Pro Thr
 25                  30                  35                  40

TCT CAG CCG AAT AGC TCC AAG CAA ACT GTC CTG TCT TGG CAA GCT GCA      678
Ser Gln Pro Asn Ser Ser Lys Gln Thr Val Leu Ser Trp Gln Ala Ala
                 45                  50                  55

ATC GAT GCT GCT AGA CAG GCC AAG GCT GCC CAA ACT ATG AGC ACC TCT      726
Ile Asp Ala Ala Arg Gln Ala Lys Ala Ala Gln Thr Met Ser Thr Ser
             60                  65                  70

GCA CCC CCA CCT GTA GGA TCT CTC TCC CAA AGA AAA CGT CAG CAA TAC      774
Ala Pro Pro Pro Val Gly Ser Leu Ser Gln Arg Lys Arg Gln Gln Tyr
         75                  80                  85
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GCC AAG AGC AAA AAA CAG GGT AAC TCG TCC AAC AGC CGA CCT GCC CGC          822
Ala Lys Ser Lys Lys Gln Gly Asn Ser Ser Asn Ser Arg Pro Ala Arg
     90                  95                  100

GCC CTT TTC TGT TTA TCA CTC AAT AAC CCC ATC CGA AGA GCC TGC ATT          870
Ala Leu Phe Cys Leu Ser Leu Asn Asn Pro Ile Arg Arg Ala Cys Ile
105                 110                 115                 120

AGT ATA GTG GAA TGG AAA CCA TTT GAC ATA TTT ATA TTA TTG GCT ATT          918
Ser Ile Val Glu Trp Lys Pro Phe Asp Ile Phe Ile Leu Leu Ala Ile
                125                 130                 135

TTT GCC AAT TGT GTG GCC TTA GCT ATT TAC ATC CCA TTC CCT GAA GAT          966
Phe Ala Asn Cys Val Ala Leu Ala Ile Tyr Ile Pro Phe Pro Glu Asp
            140                 145                 150

GAT TCT AAT TCA ACA AAT CAT AAC TTG GAA AAA GTA GAA TAT GCC TTC         1014
Asp Ser Asn Ser Thr Asn His Asn Leu Glu Lys Val Glu Tyr Ala Phe
        155                 160                 165

CTG ATT ATT TTT ACA GTC GAG ACA TTT TTG AAG ATT ATA GCG TAT GGA         1062
Leu Ile Ile Phe Thr Val Glu Thr Phe Leu Lys Ile Ile Ala Tyr Gly
    170                 175                 180

TTA TTG CTA CAT CCT AAT GCT TAT GTT AGG AAT GGA TGG AAT TTA CTG         1110
Leu Leu Leu His Pro Asn Ala Tyr Val Arg Asn Gly Trp Asn Leu Leu
185                 190                 195                 200

GAT TTT GTT ATA GTA ATA GTA GGA TTG TTT AGT GTA ATT TTG GAA CAA         1158
Asp Phe Val Ile Val Ile Val Gly Leu Phe Ser Val Ile Leu Glu Gln
                205                 210                 215

TTA ACC AAA GAA ACA GAA GGC GGG AAC CAC TCA AGC GGC AAA TCT GGA         1206
Leu Thr Lys Glu Thr Glu Gly Gly Asn His Ser Ser Gly Lys Ser Gly
            220                 225                 230

GGC TTT GAT GTC AAA GCC CTC CGT GCC TTT CGA GTG TTG CGA CCA CTT         1254
Gly Phe Asp Val Lys Ala Leu Arg Ala Phe Arg Val Leu Arg Pro Leu
        235                 240                 245

CGA CTA GTG TCA GGA GTG CCC AGT TTA CAA GTT GTC CTG AAC TCC ATT         1302
Arg Leu Val Ser Gly Val Pro Ser Leu Gln Val Val Leu Asn Ser Ile
    250                 255                 260

ATA AAA GCC ATG GTT CCC CTC CTT CAC ATA GCC CTT TTG GTA TTA TTT         1350
Ile Lys Ala Met Val Pro Leu Leu His Ile Ala Leu Leu Val Leu Phe
265                 270                 275                 280

GTA ATC ATA ATC TAT GCT ATT ATA GGA TTG GAA CTT TTT ATT GGA AAA         1398
Val Ile Ile Ile Tyr Ala Ile Ile Gly Leu Glu Leu Phe Ile Gly Lys
                285                 290                 295

ATG CAC AAA ACA TGT TTT TTT GCT GAC TCA GAT ATC GTA GCT GAA GAG         1446
Met His Lys Thr Cys Phe Phe Ala Asp Ser Asp Ile Val Ala Glu Glu
            300                 305                 310

GAC CCA GCT CCA TGT GCG TTC TCA GGG AAT GGA CGC CAG TGT ACT GCC         1494
Asp Pro Ala Pro Cys Ala Phe Ser Gly Asn Gly Arg Gln Cys Thr Ala
        315                 320                 325

AAT GGC ACG GAA TGT AGG AGT GGC TGG GTT GGC CCG AAC GGA GGC ATC         1542
Asn Gly Thr Glu Cys Arg Ser Gly Trp Val Gly Pro Asn Gly Gly Ile
    330                 335                 340

ACC AAC TTT GAT AAC TTT GCC TTT GCC ATG CTT ACT GTG TTT CAG TGC         1590
Thr Asn Phe Asp Asn Phe Ala Phe Ala Met Leu Thr Val Phe Gln Cys
345                 350                 355                 360
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ATC ACC ATG GAG GGC TGG ACA GAC GTG CTC TAC TGG ATG AAT GAT GCT      1638
Ile Thr Met Glu Gly Trp Thr Asp Val Leu Tyr Trp Met Asn Asp Ala
                365                 370                 375

ATG GGA TTT GAA TTG CCC TGG GTG TAT TTT GTC AGT CTC GTC ATC TTT      1686
Met Gly Phe Glu Leu Pro Trp Val Tyr Phe Val Ser Leu Val Ile Phe
            380                 385                 390

GGG TCA TTT TTC GTA CTA AAT CTT GTA CTT GGT GTA TTG AGC GGA GAA      1734
Gly Ser Phe Phe Val Leu Asn Leu Val Leu Gly Val Leu Ser Gly Glu
        395                 400                 405

TTC TCA AAG GAA AGA GAG AAG GCA AAA GCA CGG GGA GAT TTC CAG AAG      1782
Phe Ser Lys Glu Arg Glu Lys Ala Lys Ala Arg Gly Asp Phe Gln Lys
    410                 415                 420

CTC CGG GAG AAG CAG CAG CTG GAG GAG GAT CTA AAG GGC TAC TTG GAT      1830
Leu Arg Glu Lys Gln Gln Leu Glu Glu Asp Leu Lys Gly Tyr Leu Asp
425                 430                 435                 440

TGG ATC ACC CAA GCT GAG GAC ATC GAT CCG GAG AAT GAG GAA GAA GGA      1878
Trp Ile Thr Gln Ala Glu Asp Ile Asp Pro Glu Asn Glu Glu Glu Gly
                445                 450                 455

GGA GAG GAA GGC AAA CGA AAT ACT AGC ATG CCC ACC AGC GAG ACT GAG      1926
Gly Glu Glu Gly Lys Arg Asn Thr Ser Met Pro Thr Ser Glu Thr Glu
            460                 465                 470

TCT GTG AAC ACA GAG AAC GTC AGC GGT GAA GGC GAG AAC CGA GGC TGC      1974
Ser Val Asn Thr Glu Asn Val Ser Gly Glu Gly Glu Asn Arg Gly Cys
        475                 480                 485

TGT GGA AGT CTC TGT CAA GCC ATC TCA AAA TCC AAA CTC AGC CGA CGC      2022
Cys Gly Ser Leu Cys Gln Ala Ile Ser Lys Ser Lys Leu Ser Arg Arg
    490                 495                 500

TGG CGT CGC TGG AAC CGA TTC AAT CGC AGA AGA TGT AGG GCC GCC GTG      2070
Trp Arg Arg Trp Asn Arg Phe Asn Arg Arg Arg Cys Arg Ala Ala Val
505                 510                 515                 520

AAG TCT GTC ACG TTT TAC TGG CTG GTT ATC GTC CTG GTG TTT CTG AAC      2118
Lys Ser Val Thr Phe Tyr Trp Leu Val Ile Val Leu Val Phe Leu Asn
                525                 530                 535

ACC TTA ACC ATT TCC TCT GAG CAC TAC AAT CAG CCA GAT TGG TTG ACA      2166
Thr Leu Thr Ile Ser Ser Glu His Tyr Asn Gln Pro Asp Trp Leu Thr
            540                 545                 550

CAG ATT CAA GAT ATT GCC AAC AAA GTC CTC TTG GCT CTG TTC ACC TGC      2214
Gln Ile Gln Asp Ile Ala Asn Lys Val Leu Leu Ala Leu Phe Thr Cys
        555                 560                 565

GAG ATG CTG GTA AAA ATG TAC AGC TTG GGC CTC CAA GCA TAT TTC GTC      2262
Glu Met Leu Val Lys Met Tyr Ser Leu Gly Leu Gln Ala Tyr Phe Val
    570                 575                 580

TCT CTT TTC AAC CGG TTT GAT TGC TTC GTG GTG TGT GGT GGA ATC ACT      2310
Ser Leu Phe Asn Arg Phe Asp Cys Phe Val Val Cys Gly Gly Ile Thr
585                 590                 595                 600

GAG ACG ATC TTG GTG GAA CTG GAA ATC ATG TCT CCC CTG GGG ATC TCT      2358
Glu Thr Ile Leu Val Glu Leu Glu Ile Met Ser Pro Leu Gly Ile Ser
                605                 610                 615

GTG TTT CGG TGT GTG CGC CTC TTA AGA ATC TTC AAA GTG ACC AGG CAC      2406
Val Phe Arg Cys Val Arg Leu Leu Arg Ile Phe Lys Val Thr Arg His
            620                 625                 630
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TGG ACT TCC CTG AGC AAC TTA GTG GCA TCC TTA TTA AAC TCC ATG AAG          2454
Trp Thr Ser Leu Ser Asn Leu Val Ala Ser Leu Leu Asn Ser Met Lys
        635                 640                 645

TCC ATC GCT TCG CTG TTG CTT CTG CTT TTT CTC TTC ATT ATC ATC TTT          2502
Ser Ile Ala Ser Leu Leu Leu Leu Leu Phe Leu Phe Ile Ile Ile Phe
    650                 655                 660

TCC TTG CTT GGG ATG CAG CTG TTT GGC GGC AAG TTT AAT TTT GAT GAA          2550
Ser Leu Leu Gly Met Gln Leu Phe Gly Gly Lys Phe Asn Phe Asp Glu
665                 670                 675                 680

ACG CAA ACC AAG CGG AGC ACC TTT GAC AAT TTC CCT CAA GCA CTT CTC          2598
Thr Gln Thr Lys Arg Ser Thr Phe Asp Asn Phe Pro Gln Ala Leu Leu
                685                 690                 695

ACA GTG TTC CAG ATC CTG ACA GGC GAA GAC TGG AAT GCT GTG ATG TAC          2646
Thr Val Phe Gln Ile Leu Thr Gly Glu Asp Trp Asn Ala Val Met Tyr
            700                 705                 710

GAT GGC ATC ATG GCT TAC GGG GGC CCA TCC TCT TCA GGA ATG ATC GTC          2694
Asp Gly Ile Met Ala Tyr Gly Gly Pro Ser Ser Ser Gly Met Ile Val
        715                 720                 725

TGC ATC TAC TTC ATC ATC CTC TTC ATT TGT GGT AAC TAT ATT CTA CTG          2742
Cys Ile Tyr Phe Ile Ile Leu Phe Ile Cys Gly Asn Tyr Ile Leu Leu
    730                 735                 740

AAT GTC TTC TTG GCC ATC GCT GTA GAC AAT TTG GCT GAT GCT GAA AGT          2790
Asn Val Phe Leu Ala Ile Ala Val Asp Asn Leu Ala Asp Ala Glu Ser
745                 750                 755                 760

CTG AAC ACT GCT CAG AAA GAA GAA GCG GAA GAA AAG GAG AGG AAA AAG          2838
Leu Asn Thr Ala Gln Lys Glu Glu Ala Glu Glu Lys Glu Arg Lys Lys
                765                 770                 775

ATT GCC AGA AAA GAG AGC CTA GAA AAT AAA AAG AAC AAC AAA CCA GAA          2886
Ile Ala Arg Lys Glu Ser Leu Glu Asn Lys Lys Asn Asn Lys Pro Glu
            780                 785                 790

GTC AAC CAG ATA GCC AAC AGT GAC AAC AAG GTT ACA ATT GAT GAC TAT          2934
Val Asn Gln Ile Ala Asn Ser Asp Asn Lys Val Thr Ile Asp Asp Tyr
        795                 800                 805

AGA GAA GAG GAT GAA GAC AAG GAC CCC TAT CCG CCT TGC GAT GTG CCA          2982
Arg Glu Glu Asp Glu Asp Lys Asp Pro Tyr Pro Pro Cys Asp Val Pro
    810                 815                 820

GTA GGG GAA GAG GAA GAG GAA GAG GAG GAG GAT GAA CCT GAG GTT CCT          3030
Val Gly Glu Glu Glu Glu Glu Glu Glu Glu Asp Glu Pro Glu Val Pro
825                 830                 835                 840

GCC GGA CCC CGT CCT CGA AGG ATC TCG GAG TTG AAC ATG AAG GAA AAA          3078
Ala Gly Pro Arg Pro Arg Arg Ile Ser Glu Leu Asn Met Lys Glu Lys
                845                 850                 855

ATT GCC CCC ATC CCT GAA GGG AGC GCT TTC TTC ATT CTT AGC AAG ACC          3126
Ile Ala Pro Ile Pro Glu Gly Ser Ala Phe Phe Ile Leu Ser Lys Thr
            860                 865                 870

AAC CCG ATC CGC GTA GGC TGC CAC AAG CTC ATC AAC CAC CAC ATC TTC          3174
Asn Pro Ile Arg Val Gly Cys His Lys Leu Ile Asn His His Ile Phe
        875                 880                 885

ACC AAC CTC ATC CTT GTC TTC ATC ATG CTG AGC AGT GCT GCC CTG GCC          3222
Thr Asn Leu Ile Leu Val Phe Ile Met Leu Ser Ser Ala Ala Leu Ala
    890                 895                 900
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GCA GAG GAC CCC ATC CGC AGC CAC TCC TTC CGG AAC ACG ATA CTG GGT 3270
Ala Glu Asp Pro Ile Arg Ser His Ser Phe Arg Asn Thr Ile Leu Gly
905 910 915 920

TAC TTT GAC TAT GCC TTC ACA GCC ATC TTT ACT GTT GAG ATC CTG TTG 3318
Tyr Phe Asp Tyr Ala Phe Thr Ala Ile Phe Thr Val Glu Ile Leu Leu
925 930 935

AAG ATG ACA ACT TTT GGA GCT TTC CTC CAC AAA GGG GCC TTC TGC AGG 3366
Lys Met Thr Thr Phe Gly Ala Phe Leu His Lys Gly Ala Phe Cys Arg
940 945 950

AAC TAC TTC AAT TTG CTG GAT ATG CTG GTG GTT GGG GTG TCT CTG GTG 3414
Asn Tyr Phe Asn Leu Leu Asp Met Leu Val Val Gly Val Ser Leu Val
955 960 965

TCA TTT GGG ATT CAA TCC AGT GCC ATC TCC GTT GTG AAG ATT CTG AGG 3462
Ser Phe Gly Ile Gln Ser Ser Ala Ile Ser Val Val Lys Ile Leu Arg
970 975 980

GTC TTA AGG GTC CTG CGT CCC CTC AGG GCC ATC AAC AGA GCA AAA GGA 3510
Val Leu Arg Val Leu Arg Pro Leu Arg Ala Ile Asn Arg Ala Lys Gly
985 990 995 1000

CTT AAG CAC GTG GTC CAG TGC GTC TTC GTG GCC ATC CGG ACC ATC GGC 3558
Leu Lys His Val Val Gln Cys Val Phe Val Ala Ile Arg Thr Ile Gly
1005 1010 1015

AAC ATC ATG ATC GTC ACC ACC CTC CTG CAG TTC ATG TTT GCC TGT ATC 3606
Asn Ile Met Ile Val Thr Thr Leu Leu Gln Phe Met Phe Ala Cys Ile
1020 1025 1030

GGG GTC CAG TTG TTC AAG GGG AAG TTC TAT CGC TGT ACG GAT GAA GCC 3654
Gly Val Gln Leu Phe Lys Gly Lys Phe Tyr Arg Cys Thr Asp Glu Ala
1035 1040 1045

AAA AGT AAC CCT GAA GAA TGC AGG GGA CTT TTC ATC CTC TAC AAG GAT 3702
Lys Ser Asn Pro Glu Glu Cys Arg Gly Leu Phe Ile Leu Tyr Lys Asp
1050 1055 1060

GGG GAT GTT GAC AGT CCT GTG GTC CGT GAA CGG ATC TGG CAA AAC AGT 3750
Gly Asp Val Asp Ser Pro Val Val Arg Glu Arg Ile Trp Gln Asn Ser
1065 1070 1075 1080

GAT TTC AAC TTC GAC AAC GTC CTC TCT GCT ATG ATG GCG CTC TTC ACA 3798
Asp Phe Asn Phe Asp Asn Val Leu Ser Ala Met Met Ala Leu Phe Thr
1085 1090 1095

GTC TCC ACG TTT GAG GGC TGG CCT GCG TTG CTG TAT AAA GCC ATC GAC 3846
Val Ser Thr Phe Glu Gly Trp Pro Ala Leu Leu Tyr Lys Ala Ile Asp
1100 1105 1110

TCG AAT GGA GAG AAC ATC GGC CCA ATC TAC AAC CAC CGC GTG GAG ATC 3894
Ser Asn Gly Glu Asn Ile Gly Pro Ile Tyr Asn His Arg Val Glu Ile
1115 1120 1125

TCC ATC TTC TTC ATC ATC TAC ATC ATC ATT GTA GCT TTC TTC ATG ATG 3942
Ser Ile Phe Phe Ile Ile Tyr Ile Ile Ile Val Ala Phe Phe Met Met
1130 1135 1140

AAC ATC TTT GTG GGC TTT GTC ATC GTT ACA TTT CAG GAA CAA GGA GAA 3990
Asn Ile Phe Val Gly Phe Val Ile Val Thr Phe Gln Glu Gln Gly Glu
1145 1150 1155 1160

AAA GAG TAT AAG AAC TGT GAG CTG GAC AAA AAT CAG CGT CAG TGT GTT 4038
Lys Glu Tyr Lys Asn Cys Glu Leu Asp Lys Asn Gln Arg Gln Cys Val
1165 1170 1175

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GAA TAC GCC TTG AAA GCA CGT CCC TTG CGG AGA TAC ATC CCC AAA AAC      4086
Glu Tyr Ala Leu Lys Ala Arg Pro Leu Arg Arg Tyr Ile Pro Lys Asn
            1180                1185                1190

CCC TAC CAG TAC AAG TTC TGG TAC GTG GTG AAC TCT TCG CCT TTC GAA      4134
Pro Tyr Gln Tyr Lys Phe Trp Tyr Val Val Asn Ser Ser Pro Phe Glu
        1195                1200                1205

TAC ATG ATG TTT GTC CTC ATC ATG CTC AAC ACA CTC TGC TTG GCC ATG      4182
Tyr Met Met Phe Val Leu Ile Met Leu Asn Thr Leu Cys Leu Ala Met
    1210                1215                1220

CAG CAC TAC GAG CAG TCC AAG ATG TTC AAT GAT GCC ATG GAC ATT CTG      4230
Gln His Tyr Glu Gln Ser Lys Met Phe Asn Asp Ala Met Asp Ile Leu
1225                1230                1235                1240

AAC ATG GTC TTC ACC GGG GTG TTC ACC GTC GAG ATG GTT TTG AAA GTC      4278
Asn Met Val Phe Thr Gly Val Phe Thr Val Glu Met Val Leu Lys Val
                1245                1250                1255

ATC GCA TTT AAG CCT AAG GGG TAT TTT AGT GAC GCC TGG AAC ACG TTT      4326
Ile Ala Phe Lys Pro Lys Gly Tyr Phe Ser Asp Ala Trp Asn Thr Phe
            1260                1265                1270

GAC TCC CTC ATC GTA ATC GGC AGC ATT ATA GAC GTG GCC CTC AGC GAA      4374
Asp Ser Leu Ile Val Ile Gly Ser Ile Ile Asp Val Ala Leu Ser Glu
        1275                1280                1285

GCA GAC CCA ACT GAA AGT GAA AAT GTC CCT GTC CCA ACT GCT ACA CCT      4422
Ala Asp Pro Thr Glu Ser Glu Asn Val Pro Val Pro Thr Ala Thr Pro
    1290                1295                1300

GGG AAC TCT GAA GAG AGC AAT AGA ATC TCC ATC ACC TTT TTC CGT CTT      4470
Gly Asn Ser Glu Glu Ser Asn Arg Ile Ser Ile Thr Phe Phe Arg Leu
1305                1310                1315                1320

TTC CGA GTG ATG CGA TTG GTG AAG CTT CTC AGC AGG GGG GAA GGC ATC      4518
Phe Arg Val Met Arg Leu Val Lys Leu Leu Ser Arg Gly Glu Gly Ile
                1325                1330                1335

CGG ACA TTG CTG TGG ACT TTT ATT AAG TTC TTT CAG GCG CTC CCG TAT      4566
Arg Thr Leu Leu Trp Thr Phe Ile Lys Phe Phe Gln Ala Leu Pro Tyr
            1340                1345                1350

GTG GCC CTC CTC ATA GCC ATG CTG TTC TTC ATC TAT GCG GTC ATT GGC      4614
Val Ala Leu Leu Ile Ala Met Leu Phe Phe Ile Tyr Ala Val Ile Gly
        1355                1360                1365

ATG CAG ATG TTT GGG AAA GTT GCC ATG AGA GAT AAC AAC CAG ATC AAT      4662
Met Gln Met Phe Gly Lys Val Ala Met Arg Asp Asn Asn Gln Ile Asn
    1370                1375                1380

AGG AAC AAT AAC TTC CAG ACG TTT CCC CAG GCG GTG CTG CTG CTC TTC      4710
Arg Asn Asn Asn Phe Gln Thr Phe Pro Gln Ala Val Leu Leu Leu Phe
1385                1390                1395                1400

AGG TGT GCA ACA GGT GAG GCC TGG CAG GAG ATC ATG CTG GCC TGT CTC      4758
Arg Cys Ala Thr Gly Glu Ala Trp Gln Glu Ile Met Leu Ala Cys Leu
                1405                1410                1415

CCA GGG AAG CTC TGT GAC CCT GAG TCA GAT TAC AAC CCC GGG GAG GAG      4806
Pro Gly Lys Leu Cys Asp Pro Glu Ser Asp Tyr Asn Pro Gly Glu Glu
            1420                1425                1430

CAT ACA TGT GGG AGC AAC TTT GCC ATT GTC TAT TTC ATC AGT TTT TAC      4854
His Thr Cys Gly Ser Asn Phe Ala Ile Val Tyr Phe Ile Ser Phe Tyr
        1435                1440                1445
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ATG CTC TGT GCA TTT CTG ATC ATC AAT CTG TTT GTG GCT GTC ATC ATG 4902
Met Leu Cys Ala Phe Leu Ile Ile Asn Leu Phe Val Ala Val Ile Met
1450 1455 1460

GAT AAT TTC GAC TAT CTG ACC CGG GAC TGG TCT ATT TTG GGG CCT CAC 4950
Asp Asn Phe Asp Tyr Leu Thr Arg Asp Trp Ser Ile Leu Gly Pro His
1465 1470 1475 1480

CAT TTA GAT GAA TTC AAA AGA ATA TGG TCA GAA TAT GAC CCT GAG GCA 4998
His Leu Asp Glu Phe Lys Arg Ile Trp Ser Glu Tyr Asp Pro Glu Ala
1485 1490 1495

AAG GGA AGG ATA AAA CAC CTT GAT GTG GTC ACT CTG CTT CGA CGC ATC 5046
Lys Gly Arg Ile Lys His Leu Asp Val Val Thr Leu Leu Arg Arg Ile
1500 1505 1510

CAG CCT CCC CTG GGG TTT GGG AAG TTA TGT CCA CAC AGG GTA GCG TGC 5094
Gln Pro Pro Leu Gly Phe Gly Lys Leu Cys Pro His Arg Val Ala Cys
1515 1520 1525

AAG AGA TTA GTT GCC ATG AAC ATG CCT CTC AAC AGT GAC GGG ACA GTC 5142
Lys Arg Leu Val Ala Met Asn Met Pro Leu Asn Ser Asp Gly Thr Val
1530 1535 1540

ATG TTT AAT GCA ACC CTG TTT GCT TTG GTT CGA ACG GCT CTT AAG ATC 5190
Met Phe Asn Ala Thr Leu Phe Ala Leu Val Arg Thr Ala Leu Lys Ile
1545 1550 1555 1560

AAG ACC GAA GGG AAC CTG GAG CAA GCT AAT GAA GAA CTT CGG GCT GTG 5238
Lys Thr Glu Gly Asn Leu Glu Gln Ala Asn Glu Glu Leu Arg Ala Val
1565 1570 1575

ATA AAG AAA ATT TGG AAG AAA ACC AGC ATG AAA TTA CTT GAC CAA GTT 5286
Ile Lys Lys Ile Trp Lys Lys Thr Ser Met Lys Leu Leu Asp Gln Val
1580 1585 1590

GTC CCT CCA GCT GGT GAT GAT GAG GTA ACC GTG GGG AAG TTC TAT GCC 5334
Val Pro Pro Ala Gly Asp Asp Glu Val Thr Val Gly Lys Phe Tyr Ala
1595 1600 1605

ACT TTC CTG ATA CAG GAC TAC TTT AGG AAA TTC AAG AAA CGG AAA GAA 5382
Thr Phe Leu Ile Gln Asp Tyr Phe Arg Lys Phe Lys Lys Arg Lys Glu
1610 1615 1620

CAA GGA CTG GTG GGA AAG TAC CCT GCG AAG AAC ACC ACA ATT GCC CTA 5430
Gln Gly Leu Val Gly Lys Tyr Pro Ala Lys Asn Thr Thr Ile Ala Leu
1625 1630 1635 1640

CAG GCG GGA TTA AGG ACA CTG CAT GAC ATT GGG CCA GAA ATC CGG CGT 5478
Gln Ala Gly Leu Arg Thr Leu His Asp Ile Gly Pro Glu Ile Arg Arg
1645 1650 1655

GCT ATA TCG TGT GAT TTG CAA GAT GAC GAG CCT GAG GAA ACA AAA CGA 5526
Ala Ile Ser Cys Asp Leu Gln Asp Asp Glu Pro Glu Glu Thr Lys Arg
1660 1665 1670

GAA GAA GAA GAT GAT GTG TTC AAA AGA AAT GGT GCC CTG CTT GGA AAC 5574
Glu Glu Glu Asp Asp Val Phe Lys Arg Asn Gly Ala Leu Leu Gly Asn
1675 1680 1685

CAT GTC AAT CAT GTT AAT AGT GAT AGG AGA GAT TCC CTT CAG CAG ACC 5622
His Val Asn His Val Asn Ser Asp Arg Arg Asp Ser Leu Gln Gln Thr
1690 1695 1700

AAT ACC ACC CAC CGT CCC CTG CAT GTC CAA AGG CCT TCA ATT CCA CCT 5670
Asn Thr Thr His Arg Pro Leu His Val Gln Arg Pro Ser Ile Pro Pro
1705 1710 1715 1720

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GCA AGT GAT ACT GAG AAA CCG CTG TTT CCT CCA GCA GGA AAT TCG GTG      5718
Ala Ser Asp Thr Glu Lys Pro Leu Phe Pro Pro Ala Gly Asn Ser Val
                1725                1730                1735

TGT CAT AAC CAT CAT AAC CAT AAT TCC ATA GGA AAG CAA GTT CCC ACC      5766
Cys His Asn His His Asn His Asn Ser Ile Gly Lys Gln Val Pro Thr
            1740                1745                1750

TCA ACA AAT GCC AAT CTC AAT AAT GCC AAT ATG TCC AAA GCT GCC CAT      5814
Ser Thr Asn Ala Asn Leu Asn Asn Ala Asn Met Ser Lys Ala Ala His
        1755                1760                1765

GGA AAG CGG CCC AGC ATT GGG AAC CTT GAG CAT GTG TCT GAA AAT GGG      5862
Gly Lys Arg Pro Ser Ile Gly Asn Leu Glu His Val Ser Glu Asn Gly
    1770                1775                1780

CAT CAT TCT TCC CAC AAG CAT GAC CGG GAG CCT CAG AGA AGG TCC AGT      5910
His His Ser Ser His Lys His Asp Arg Glu Pro Gln Arg Arg Ser Ser
1785                1790                1795                1800

GTG AAA AGA ACC CGC TAT TAT GAA ACT TAC ATT AGG TCC GAC TCA GGA      5958
Val Lys Arg Thr Arg Tyr Tyr Glu Thr Tyr Ile Arg Ser Asp Ser Gly
                1805                1810                1815

GAT GAA CAG CTC CCA ACT ATT TGC CGG GAA GAC CCA GAG ATA CAT GGC      6006
Asp Glu Gln Leu Pro Thr Ile Cys Arg Glu Asp Pro Glu Ile His Gly
            1820                1825                1830

TAT TTC AGG GAC CCC CAC TGC TTG GGG GAG CAG GAG TAT TTC AGT AGT      6054
Tyr Phe Arg Asp Pro His Cys Leu Gly Glu Gln Glu Tyr Phe Ser Ser
        1835                1840                1845

GAG GAA TGC TAC GAG GAT GAC AGC TCG CCC ACC TGG AGC AGG CAA AAC      6102
Glu Glu Cys Tyr Glu Asp Asp Ser Ser Pro Thr Trp Ser Arg Gln Asn
    1850                1855                1860

TAT GGC TAC TAC AGC AGA TAC CCA GGC AGA AAC ATC GAC TCT GAG AGG      6150
Tyr Gly Tyr Tyr Ser Arg Tyr Pro Gly Arg Asn Ile Asp Ser Glu Arg
1865                1870                1875                1880

CCC CGA GGC TAC CAT CAT CCC CAA GGA TTC TTG GAG GAC GAT GAC TCG      6198
Pro Arg Gly Tyr His His Pro Gln Gly Phe Leu Glu Asp Asp Asp Ser
                1885                1890                1895

CCC GTT TGC TAT GAT TCA CGG AGA TCT CCA AGG AGA CGC CTA CTA CCT      6246
Pro Val Cys Tyr Asp Ser Arg Arg Ser Pro Arg Arg Arg Leu Leu Pro
            1900                1905                1910

CCC ACC CCA GCA TCC CAC CGG AGA TCC TCC TTC AAC TTT GAG TGC CTG      6294
Pro Thr Pro Ala Ser His Arg Arg Ser Ser Phe Asn Phe Glu Cys Leu
        1915                1920                1925

CGC CGG CAG AGC AGC CAG GAA GAG GTC CCG TCG TCT CCC ATC TTC CCC      6342
Arg Arg Gln Ser Ser Gln Glu Glu Val Pro Ser Ser Pro Ile Phe Pro
    1930                1935                1940

CAT CGC ACG GCC CTG CCT CTG CAT CTA ATG CAG CAA CAG ATC ATG GCA      6390
His Arg Thr Ala Leu Pro Leu His Leu Met Gln Gln Gln Ile Met Ala
1945                1950                1955                1960

GTT GCC GGC CTA GAT TCA AGT AAA GCC CAG AAG TAC TCA CCG AGT CAC      6438
Val Ala Gly Leu Asp Ser Ser Lys Ala Gln Lys Tyr Ser Pro Ser His
                1965                1970                1975

TCG ACC CGG TCG TGG GCC ACC CCT CCA GCA ACC CCT CCC TAC CGG GAC      6486
Ser Thr Arg Ser Trp Ala Thr Pro Pro Ala Thr Pro Pro Tyr Arg Asp
            1980                1985                1990
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TGG ACA CCG TGC TAC ACC CCC CTG ATC CAA GTG GAG CAG TCA GAG GCC          6534
Trp Thr Pro Cys Tyr Thr Pro Leu Ile Gln Val Glu Gln Ser Glu Ala
        1995                2000                2005

CTG GAC CAG GTG AAC GGC AGC CTG CCG TCC CTG CAC CGC AGC TCC TGG          6582
Leu Asp Gln Val Asn Gly Ser Leu Pro Ser Leu His Arg Ser Ser Trp
    2010                2015                2020

TAC ACA GAC GAG CCC GAC ATC TCC TAC CGG ACT TTC ACA CCA GCC AGC          6630
Tyr Thr Asp Glu Pro Asp Ile Ser Tyr Arg Thr Phe Thr Pro Ala Ser
2025                2030                2035                2040

CTG ACT GTC CCC AGC AGC TTC CGG AAC AAA AAC AGC GAC AAG CAG AGG          6678
Leu Thr Val Pro Ser Ser Phe Arg Asn Lys Asn Ser Asp Lys Gln Arg
                2045                2050                2055

AGT GCG GAC AGC TTG GTG GAG GCA GTC CTG ATA TCC GAA GGC TTG GGA          6726
Ser Ala Asp Ser Leu Val Glu Ala Val Leu Ile Ser Glu Gly Leu Gly
            2060                2065                2070

CGC TAT GCA AGG GAC CCA AAA TTT GTG TCA GCA ACA AAA CAC GAA ATC          6774
Arg Tyr Ala Arg Asp Pro Lys Phe Val Ser Ala Thr Lys His Glu Ile
        2075                2080                2085

GCT GAT GCC TGT GAC CTC ACC ATC GAC GAG ATG GAG AGT GCA GCC AGC          6822
Ala Asp Ala Cys Asp Leu Thr Ile Asp Glu Met Glu Ser Ala Ala Ser
    2090                2095                2100

ACC CTG CTT AAT GGG AAC GTG CGT CCC CGA GCC AAC GGG GAT GTG GGC          6870
Thr Leu Leu Asn Gly Asn Val Arg Pro Arg Ala Asn Gly Asp Val Gly
2105                2110                2115                2120

CCC CTC TCA CAC CGG CAG GAC TAT GAG CTA CAG GAC TTT GGT CCT GGC          6918
Pro Leu Ser His Arg Gln Asp Tyr Glu Leu Gln Asp Phe Gly Pro Gly
                2125                2130                2135

TAC AGC GAC GAA GAG CCA GAC CCT GGG AGG GAT GAG GAG GAC CTG GCG          6966
Tyr Ser Asp Glu Glu Pro Asp Pro Gly Arg Asp Glu Glu Asp Leu Ala
            2140                2145                2150

GAT GAA ATG ATA TGC ATC ACC ACC TTG TAGCCCCCAG CGAGGGGCAG                7013
Asp Glu Met Ile Cys Ile Thr Thr Leu
        2155                2160

ACTGGCTCTG GCCTCAGGTG GGGCGCAGGA GAGCCAGGGG AAAAGTGCCT CATAGTTAGG       7073

AAAGTTTAGG CACTAGTTGG GAGTAATATT CAATTAATTA GACTTTTGTA TAAGAGATGT       7133

CATGCCTCAA GAAAGCCATA AACCTGGTAG GAACAGGTCC CAAGCGGTTG AGCCTGGCAG       7193

AGTACCATGC GCTCGGCCCC AGCTGCAGGA AACAGCAGGC CCCGCCCTCT CACAGAGGAT       7253

GGGTGAGGAG GCCAGACCTG CCCTGCCCCA TTGTCCAGAT GGGCACTGCT GTGGAGTCTG       7313

CTTCTCCCAT GTACCAGGGC ACCAGGCCCA CCCAACTGAA GGCATGGCGG CGGGGTGCAG       7373

GGGAAAGTTA AAGGTGATGA CGATCATCAC ACCTGTGTCG TTACCTCAGC CATCGGTCTA       7433

GCATATCAGT CACTGGGCCC AACATATCCA TTTTTAAACC CTTTCCCCCA AATACACTGC       7493

GTCCTGGTTC CTGTTTAGCT GTTCTGAAAT ACGGTGTGTA AGTAAGTCAG AACCCAGCTA       7553

CCAGTGATTA TTGCGAGGGC AATGGGACCT CATAAATAAG GTTTTCTGTG ATGTGACGCC       7613

AGTTTACATA AGAGAATATC AC                                                7635
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 104 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..102

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..104
(D) OTHER INFORMATION: /note= "A 104-nucleotide alternative exon of alpha-1D."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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GTA AAT GAT GCG ATA GGA TGG GAA TGG CCA TGG GTG TAT TTT GTT AGT        48
Val Asn Asp Ala Ile Gly Trp Glu Trp Pro Trp Val Tyr Phe Val Ser
 1               5                  10                  15

CTG ATC ATC CTT GGC TCA TTT TTC GTC CTT AAC CTG GTT CTT GGT GTC        96
Leu Ile Ile Leu Gly Ser Phe Phe Val Leu Asn Leu Val Leu Gly Val
            20                  25                  30

CTT AGT GG                                                           104
Leu Ser
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5904 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..5904

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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ATG GTC AAT GAG AAT ACG AGG ATG TAC ATT CCA GAG GAA AAC CAC CAA        48
Met Val Asn Glu Asn Thr Arg Met Tyr Ile Pro Glu Glu Asn His Gln
 1               5                  10                  15

GGT TCC AAC TAT GGG AGC CCA CGC CCC GCC CAT GCC AAC ATG AAT GCC        96
Gly Ser Asn Tyr Gly Ser Pro Arg Pro Ala His Ala Asn Met Asn Ala
            20                  25                  30

AAT GCG GCA GCG GGG CTG GCC CCT GAG CAC ATC CCC ACC CCG GGG GCT       144
Asn Ala Ala Ala Gly Leu Ala Pro Glu His Ile Pro Thr Pro Gly Ala
        35                  40                  45

GCC CTG TCG TGG CAG GCG GCC ATC GAC GCA GCC CGG CAG GCT AAG CTG       192
Ala Leu Ser Trp Gln Ala Ala Ile Asp Ala Ala Arg Gln Ala Lys Leu
    50                  55                  60
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GAT GGT CCC AAG CAC GGC ATC ACC AAC TTT GAC AAC TTT GCC TTC GCC      1056
Asp Gly Pro Lys His Gly Ile Thr Asn Phe Asp Asn Phe Ala Phe Ala
            340                 345                 350

ATG CTC ACG GTG TTC CAG TGC ATC ACC ATG GAG GGC TGG ACG GAC GTG      1104
Met Leu Thr Val Phe Gln Cys Ile Thr Met Glu Gly Trp Thr Asp Val
        355                 360                 365

CTG TAC TGG GTC AAT GAT GCC GTA GGA AGG GAC TGG CCC TGG ATC TAT      1152
Leu Tyr Trp Val Asn Asp Ala Val Gly Arg Asp Trp Pro Trp Ile Tyr
    370                 375                 380

TTT GTT ACA CTA ATC ATC ATA GGG TCA TTT TTT GTA CTT AAC TTG GTT      1200
Phe Val Thr Leu Ile Ile Ile Gly Ser Phe Phe Val Leu Asn Leu Val
385                 390                 395                 400

CTC GGT GTG CTT AGC GGA GAG TTT TCC AAA GAG AGG GAG AAG GCC AAG      1248
Leu Gly Val Leu Ser Gly Glu Phe Ser Lys Glu Arg Glu Lys Ala Lys
                405                 410                 415

GCC CGG GGA GAT TTC CAG AAG CTG CGG GAG AAG CAG CAG CTA GAA GAG      1296
Ala Arg Gly Asp Phe Gln Lys Leu Arg Glu Lys Gln Gln Leu Glu Glu
            420                 425                 430

GAT CTC AAA GGC TAC CTG GAT TGG ATC ACT CAG GCC GAA GAC ATC GNT      1344
Asp Leu Lys Gly Tyr Leu Asp Trp Ile Thr Gln Ala Glu Asp Ile Xaa
        435                 440                 445

CCT GAG AAT GAG GAC GAA GGC ATG GAT GAG GAG AAG CCC CGA AAC AGA      1392
Pro Glu Asn Glu Asp Glu Gly Met Asp Glu Glu Lys Pro Arg Asn Arg
    450                 455                 460

GGC ACT CCG GCG GGC ATG CTT GAT CAG AAG AAA GGG AAG TTT GCT TGG      1440
Gly Thr Pro Ala Gly Met Leu Asp Gln Lys Lys Gly Lys Phe Ala Trp
465                 470                 475                 480

TTT AGT CAC TCC ACA GAA ACC CAT GTG AGC ATG CCC ACC AGT GAG ACC      1488
Phe Ser His Ser Thr Glu Thr His Val Ser Met Pro Thr Ser Glu Thr
                485                 490                 495

GAG TCC GTC AAC ACC GAA AAC GTG GCT GGA GGT GAC ATC GAG GGA GAA      1536
Glu Ser Val Asn Thr Glu Asn Val Ala Gly Gly Asp Ile Glu Gly Glu
            500                 505                 510

AAC TGC GGG GCC AGG CTG GCC CAC CGG ATC TCC AAG TCA AAG TTC AGC      1584
Asn Cys Gly Ala Arg Leu Ala His Arg Ile Ser Lys Ser Lys Phe Ser
        515                 520                 525

CGC TAC TGG CGC CGG TGG AAT CGG TTC TGC AGA AGG AAG TGC CGC GCC      1632
Arg Tyr Trp Arg Arg Trp Asn Arg Phe Cys Arg Arg Lys Cys Arg Ala
    530                 535                 540

GCA GTC AAG TCT AAT GTC TTC TAC TGG CTG GTG ATT TTC CTG GTG TTC      1680
Ala Val Lys Ser Asn Val Phe Tyr Trp Leu Val Ile Phe Leu Val Phe
545                 550                 555                 560

CTC AAC ACG CTC ACC ATT GCC TCT GAG CAC TAC AAC CAG CCC AAC TGG      1728
Leu Asn Thr Leu Thr Ile Ala Ser Glu His Tyr Asn Gln Pro Asn Trp
                565                 570                 575

CTC ACA GAA GTC CAA GAC ACG GCA AAC AAG GCC CTG CTG GCC CTG TTC      1776
Leu Thr Glu Val Gln Asp Thr Ala Asn Lys Ala Leu Leu Ala Leu Phe
            580                 585                 590

ACG GCA GAG ATG CTC CTG AAG ATG TAC AGC CTG GGC CTG CAG GCC TAC      1824
Thr Ala Glu Met Leu Leu Lys Met Tyr Ser Leu Gly Leu Gln Ala Tyr
        595                 600                 605
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TTC GTG TCC CTC TTC AAC CGC TTT GAC TGC TTC GTC GTG TGT GGC GGC          1872
Phe Val Ser Leu Phe Asn Arg Phe Asp Cys Phe Val Val Cys Gly Gly
    610                 615                 620

ATC CTG GAG ACC ATC CTG GTG GAG ACC AAG ATC ATG TCC CCA CTG GGC          1920
Ile Leu Glu Thr Ile Leu Val Glu Thr Lys Ile Met Ser Pro Leu Gly
625                 630                 635                 640

ATC TCC GTG CTC AGA TGC GTC CGG CTG CTG AGG ATT TTC AAG ATC ACG          1968
Ile Ser Val Leu Arg Cys Val Arg Leu Leu Arg Ile Phe Lys Ile Thr
                645                 650                 655

AGG TAC TGG AAC TCC TTG AGC AAC CTG GTG GCA TCC TTG CTG AAC TCT          2016
Arg Tyr Trp Asn Ser Leu Ser Asn Leu Val Ala Ser Leu Leu Asn Ser
            660                 665                 670

GTG CGC TCC ATC GCC TCC CTG CTC CTT CTC CTC TTC CTC TTC ATC ATC          2064
Val Arg Ser Ile Ala Ser Leu Leu Leu Leu Leu Phe Leu Phe Ile Ile
        675                 680                 685

ATC TTC TCC CTC CTG GGG ATG CAG CTC TTT GGA GGA AAG TTC AAC TTT          2112
Ile Phe Ser Leu Leu Gly Met Gln Leu Phe Gly Gly Lys Phe Asn Phe
    690                 695                 700

GAT GAG ATG CAG ACC CGG AGG AGC ACA TTC GAT AAC TTC CCC CAG TCC          2160
Asp Glu Met Gln Thr Arg Arg Ser Thr Phe Asp Asn Phe Pro Gln Ser
705                 710                 715                 720

CTC CTC ACT GTG TTT CAG ATC CTG ACC GGG GAG GAC TGG AAT TCG GTG          2208
Leu Leu Thr Val Phe Gln Ile Leu Thr Gly Glu Asp Trp Asn Ser Val
                725                 730                 735

ATG TAT GAT GGG ATC ATG GCT TAT GGG GGC CCC TCT TTT CCA GGG ATG          2256
Met Tyr Asp Gly Ile Met Ala Tyr Gly Gly Pro Ser Phe Pro Gly Met
            740                 745                 750

TTA GTC TGT ATT TAC TTC ATC ATC CTC TTC ATC TCT GGA AAC TAT ATC          2304
Leu Val Cys Ile Tyr Phe Ile Ile Leu Phe Ile Ser Gly Asn Tyr Ile
        755                 760                 765

CTA CTG AAT GTG TTC TTG GCC ATT GCT GTG GAC AAC CTG GCT GAT GCT          2352
Leu Leu Asn Val Phe Leu Ala Ile Ala Val Asp Asn Leu Ala Asp Ala
    770                 775                 780

GAG AGC CTC ACA TCT GCC CTA AAG GAG GAG GAA GAG GAG AAG GAG AGA          2400
Glu Ser Leu Thr Ser Ala Leu Lys Glu Glu Glu Glu Glu Lys Glu Arg
785                 790                 795                 800

AAG AAG CTG GCC AGG ACT GCC AGC CCA GAG AAG AAA CAA GAG TTG GTG          2448
Lys Lys Leu Ala Arg Thr Ala Ser Pro Glu Lys Lys Gln Glu Leu Val
                805                 810                 815

GAG AAG CCG GCA GTG GGG GAA TCC AAG GAG GAG AAG ATT GAG CTG AAA          2496
Glu Lys Pro Ala Val Gly Glu Ser Lys Glu Glu Lys Ile Glu Leu Lys
            820                 825                 830

TCC ATC ACG GCT GAC GGA GAG TCT CCA CCC GCC ACC AAG ATC AAC ATG          2544
Ser Ile Thr Ala Asp Gly Glu Ser Pro Pro Ala Thr Lys Ile Asn Met
        835                 840                 845

GAT GAC CTC CAG CCC AAT GAA AAT GAG GAT AAG AGC CCC TAC CCC AAC          2592
Asp Asp Leu Gln Pro Asn Glu Asn Glu Asp Lys Ser Pro Tyr Pro Asn
    850                 855                 860

CCA GAA ACT ACA GGA GAA GAG GAT GAG GAG GAG CCA GAG ATG CCT GTC          2640
Pro Glu Thr Thr Gly Glu Glu Asp Glu Glu Glu Pro Glu Met Pro Val
865                 870                 875                 880
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GGC CCT CGC CCA CGA CCA CTC TCT GAG CTT CAC CTT AAG GAA AAG GCA      2688
Gly Pro Arg Pro Arg Pro Leu Ser Glu Leu His Leu Lys Glu Lys Ala
                885                 890                 895

GTG CCC ATG CCA GAA GCC AGC GCG TTT TTC ATC TTC AGC TCT AAC AAC      2736
Val Pro Met Pro Glu Ala Ser Ala Phe Phe Ile Phe Ser Ser Asn Asn
            900                 905                 910

AGG TTT CGC CTC CAG TGC CAC CGC ATT GTC AAT GAC ACG ATC TTC ACC      2784
Arg Phe Arg Leu Gln Cys His Arg Ile Val Asn Asp Thr Ile Phe Thr
        915                 920                 925

AAC CTG ATC CTC TTC TTC ATT CTG CTC AGC AGC ATT TCC CTG GCT GCT      2832
Asn Leu Ile Leu Phe Phe Ile Leu Leu Ser Ser Ile Ser Leu Ala Ala
    930                 935                 940

GAG GAC CCG GTC CAG CAC ACC TCC TTC AGG AAC CAT ATT CTG TTT TAT      2880
Glu Asp Pro Val Gln His Thr Ser Phe Arg Asn His Ile Leu Phe Tyr
945                 950                 955                 960

TTT GAT ATT GTT TTT ACC ACC ATT TTC ACC ATT GAA ATT GCT CTG AAG      2928
Phe Asp Ile Val Phe Thr Thr Ile Phe Thr Ile Glu Ile Ala Leu Lys
                965                 970                 975

ATG ACT GCT TAT GGG GCT TTC TTG CAC AAG GGT TCT TTC TGC CGG AAC      2976
Met Thr Ala Tyr Gly Ala Phe Leu His Lys Gly Ser Phe Cys Arg Asn
            980                 985                 990

TAC TTC AAC ATC CTG GAC CTG CTG GTG GTC AGC GTG TCC CTC ATC TCC      3024
Tyr Phe Asn Ile Leu Asp Leu Leu Val Val Ser Val Ser Leu Ile Ser
        995                 1000                1005

TTT GGC ATC CAG TCC AGT GCA ATC AAT GTC GTG AAG ATC TTG CGA GTC      3072
Phe Gly Ile Gln Ser Ser Ala Ile Asn Val Val Lys Ile Leu Arg Val
    1010                1015                1020

CTG CGA GTA CTC AGG CCC CTG AGG GCC ATC AAC AGG GCC AAG GGG CTA      3120
Leu Arg Val Leu Arg Pro Leu Arg Ala Ile Asn Arg Ala Lys Gly Leu
1025                1030                1035                1040

AAG CAT GTG GTT CAG TGT GTG TTT GTC GCC ATC CGG ACC ATC GGG AAC      3168
Lys His Val Val Gln Cys Val Phe Val Ala Ile Arg Thr Ile Gly Asn
                1045                1050                1055

ATC GTG ATT GTC ACC ACC CTG CTG CAG TTC ATG TTT GCC TGC ATC GGG      3216
Ile Val Ile Val Thr Thr Leu Leu Gln Phe Met Phe Ala Cys Ile Gly
            1060                1065                1070

GTC CAG CTC TTC AAG GGA AAG CTG TAC ACC TGT TCA GAC AGT TCC AAG      3264
Val Gln Leu Phe Lys Gly Lys Leu Tyr Thr Cys Ser Asp Ser Ser Lys
        1075                1080                1085

CAG ACA GAG GCG GAA TGC AAG GGC AAC TAC ATC ACG TAC AAA GAC GGG      3312
Gln Thr Glu Ala Glu Cys Lys Gly Asn Tyr Ile Thr Tyr Lys Asp Gly
    1090                1095                1100

GAG GTT GAC CAC CCC ATC ATC CAA CCC CGC AGC TGG GAG AAC AGC AAG      3360
Glu Val Asp His Pro Ile Ile Gln Pro Arg Ser Trp Glu Asn Ser Lys
1105                1110                1115                1120

TTT GAC TTT GAC AAT GTT CTG GCA GCC ATG ATG GCC CTC TTC ACC GTC      3408
Phe Asp Phe Asp Asn Val Leu Ala Ala Met Met Ala Leu Phe Thr Val
                1125                1130                1135

TCC ACC TTC GAA GGG TGG CCA GAG CTG CTG TAC CGC TCC ATC GAC TCC      3456
Ser Thr Phe Glu Gly Trp Pro Glu Leu Leu Tyr Arg Ser Ile Asp Ser
            1140                1145                1150
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CAC ACG GAA GAC AAG GGC CCC ATC TAC AAC TAC CGT GTG GAG ATC TCC      3504
His Thr Glu Asp Lys Gly Pro Ile Tyr Asn Tyr Arg Val Glu Ile Ser
        1155                1160                1165

ATC TTC TTC ATC ATC TAC ATC ATC ATC ATC GCC TTC TTC ATG ATG AAC      3552
Ile Phe Phe Ile Ile Tyr Ile Ile Ile Ile Ala Phe Phe Met Met Asn
    1170                1175                1180

ATC TTC GTG GGC TTC GTC ATC GTC ACC TTT CAG GAG CAG GGG GAG CAG      3600
Ile Phe Val Gly Phe Val Ile Val Thr Phe Gln Glu Gln Gly Glu Gln
1185                1190                1195                1200

GAG TAC AAG AAC TGT GAG CTG GAC AAG AAC CAG CGA CAG TGC GTG GAA      3648
Glu Tyr Lys Asn Cys Glu Leu Asp Lys Asn Gln Arg Gln Cys Val Glu
                1205                1210                1215

TAC GCC CTC AAG GCC CGG CCC CTG CGG AGG TAC ATC CCC AAG AAC CAG      3696
Tyr Ala Leu Lys Ala Arg Pro Leu Arg Arg Tyr Ile Pro Lys Asn Gln
            1220                1225                1230

CAC CAG TAC AAA GTG TGG TAC GTG GTC AAC TCC ACC TAC TTC GAG TAC      3744
His Gln Tyr Lys Val Trp Tyr Val Val Asn Ser Thr Tyr Phe Glu Tyr
        1235                1240                1245

CTG ATG TTC GTC CTC ATC CTG CTC AAC ACC ATC TGC CTG GCC ATG CAG      3792
Leu Met Phe Val Leu Ile Leu Leu Asn Thr Ile Cys Leu Ala Met Gln
    1250                1255                1260

CAC TAC GGC CAG AGC TGC CTG TTC AAA ATC GCC ATG AAC ATC CTC AAC      3840
His Tyr Gly Gln Ser Cys Leu Phe Lys Ile Ala Met Asn Ile Leu Asn
1265                1270                1275                1280

ATG CTC TTC ACT GGC CTC TTC ACC GTG GAG ATG ATC CTG AAG CTC ATT      3888
Met Leu Phe Thr Gly Leu Phe Thr Val Glu Met Ile Leu Lys Leu Ile
                1285                1290                1295

GCC TTC AAA CCC AAG GGT TAC TTT AGT GAT CCC TGG AAT GTT TTT GAC      3936
Ala Phe Lys Pro Lys Gly Tyr Phe Ser Asp Pro Trp Asn Val Phe Asp
            1300                1305                1310

TTC CTC ATC GTA ATT GGC AGC ATA ATT GAC GTC ATT CTC AGT GAG ACT      3984
Phe Leu Ile Val Ile Gly Ser Ile Ile Asp Val Ile Leu Ser Glu Thr
        1315                1320                1325

AAT CCA GCT GAA CAT ACC CAA TGC TCT CCC TCT ATG AAC GCA GAG GAA      4032
Asn Pro Ala Glu His Thr Gln Cys Ser Pro Ser Met Asn Ala Glu Glu
    1330                1335                1340

AAC TCC CGC ATC TCC ATC ACC TTC TTC CGC CTG TTC CGG GTC ATG CGT      4080
Asn Ser Arg Ile Ser Ile Thr Phe Phe Arg Leu Phe Arg Val Met Arg
1345                1350                1355                1360

CTG GTG AAG CTG CTG AGC CGT GGG GAG GGC ATC CGG ACG CTG CTG TGG      4128
Leu Val Lys Leu Leu Ser Arg Gly Glu Gly Ile Arg Thr Leu Leu Trp
                1365                1370                1375

ACC TTC ATC AAG TCC TTC CAG GCC CTG CCC TAT GTG GCC CTC CTG ATC      4176
Thr Phe Ile Lys Ser Phe Gln Ala Leu Pro Tyr Val Ala Leu Leu Ile
            1380                1385                1390

GTG ATG CTG TTC TTC ATC TAC GCG GTG ATC GGG ATG CAG GTG TTT GGG      4224
Val Met Leu Phe Phe Ile Tyr Ala Val Ile Gly Met Gln Val Phe Gly
        1395                1400                1405

AAA ATT GCC CTG AAT GAT ACC ACA GAG ATC AAC CGG AAC AAC AAC TTT      4272
Lys Ile Ala Leu Asn Asp Thr Thr Glu Ile Asn Arg Asn Asn Asn Phe
    1410                1415                1420
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CAG ACC TTC CCC CAG GCC GTG CTG CTC CTC TTC AGG TGT GCC ACC GGG      4320
Gln Thr Phe Pro Gln Ala Val Leu Leu Leu Phe Arg Cys Ala Thr Gly
1425                1430                1435                1440

GAG GCC TGG CAG GAC ATC ATG CTG GCC TGC ATG CCA GGC AAG AAG TGT      4368
Glu Ala Trp Gln Asp Ile Met Leu Ala Cys Met Pro Gly Lys Lys Cys
                1445                1450                1455

GCC CCA GAG TCC GAG CCC AGC AAC AGC ACG GAG GGT GAA ACA CCC TGT      4416
Ala Pro Glu Ser Glu Pro Ser Asn Ser Thr Glu Gly Glu Thr Pro Cys
            1460                1465                1470

GGT AGC AGC TTT GCT GTC TTC TAC TTC ATC AGC TTC TAC ATG CGC TGT      4464
Gly Ser Ser Phe Ala Val Phe Tyr Phe Ile Ser Phe Tyr Met Arg Cys
        1475                1480                1485

GCC TTC CTG ATC ATC AAC CTC TTT GTA GCT GTC ATC ATG GAC AAC TTT      4512
Ala Phe Leu Ile Ile Asn Leu Phe Val Ala Val Ile Met Asp Asn Phe
    1490                1495                1500

GAC TAC CTG ACA AGG GAC TGG TCC ATC CTT GGT CCC CAC CAC CTG GAT      4560
Asp Tyr Leu Thr Arg Asp Trp Ser Ile Leu Gly Pro His His Leu Asp
1505                1510                1515                1520

GAG TTT AAA AGA ATC TGG GCA GAG TAT GAC CCT GAA GCC AAG GGT CGT      4608
Glu Phe Lys Arg Ile Trp Ala Glu Tyr Asp Pro Glu Ala Lys Gly Arg
                1525                1530                1535

ATC AAA CAC CTG GAT GTG GTG ACC CTC CTC CGG CGG ATT CAG CCG CCA      4656
Ile Lys His Leu Asp Val Val Thr Leu Leu Arg Arg Ile Gln Pro Pro
            1540                1545                1550

CTA GGT TTT GGG AAG CTG TGC CCT CAC CGC GTG GCT TGC AAA CGC CTG      4704
Leu Gly Phe Gly Lys Leu Cys Pro His Arg Val Ala Cys Lys Arg Leu
        1555                1560                1565

GTC TCC ATG AAC ATG CCT CTG AAC AGC GAC GGG ACA GTC ATG TTC AAT      4752
Val Ser Met Asn Met Pro Leu Asn Ser Asp Gly Thr Val Met Phe Asn
    1570                1575                1580

GCC ACC CTG TTT GCC CTG GTC AGG ACG GCC CTG AGG ATC AAA ACA GAA      4800
Ala Thr Leu Phe Ala Leu Val Arg Thr Ala Leu Arg Ile Lys Thr Glu
1585                1590                1595                1600

GGG AAC CTA GAA CAA GCC AAT GAG GAG CTG CGG GCG ATC ATC AAG AAG      4848
Gly Asn Leu Glu Gln Ala Asn Glu Glu Leu Arg Ala Ile Ile Lys Lys
                1605                1610                1615

ATC TGG AAG CGG ACC AGC ATG AAG CTG CTG GAC CAG GTG GTG CCC CCT      4896
Ile Trp Lys Arg Thr Ser Met Lys Leu Leu Asp Gln Val Val Pro Pro
            1620                1625                1630

GCA GGT GAT GAT GAG GTC ACC GTT GGC AAG TTC TAC GCC ACG TTC CTG      4944
Ala Gly Asp Asp Glu Val Thr Val Gly Lys Phe Tyr Ala Thr Phe Leu
        1635                1640                1645

ATC CAG GAG TAC TTC CGG AAG TTC AAG AAG CGC AAA GAG CAG GGC CTT      4992
Ile Gln Glu Tyr Phe Arg Lys Phe Lys Lys Arg Lys Glu Gln Gly Leu
    1650                1655                1660

GTG GGC AAG CCC TCC CAG AGG AAC GCG CTG TCT CTG CAG GCT GGC TTG      5040
Val Gly Lys Pro Ser Gln Arg Asn Ala Leu Ser Leu Gln Ala Gly Leu
1665                1670                1675                1680

CGC ACA CTG CAT GAC ATC GGG CCT GAG ATC CGA CGG GCC ATC TCT GGA      5088
Arg Thr Leu His Asp Ile Gly Pro Glu Ile Arg Arg Ala Ile Ser Gly
                1685                1690                1695
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GAT CTC ACC GCT GAG GAG GAG CTG GAC AAG GCC ATG AAG GAG GCT GTG      5136
Asp Leu Thr Ala Glu Glu Glu Leu Asp Lys Ala Met Lys Glu Ala Val
            1700                1705                1710

TCC GCT GCT TCT GAA GAT GAC ATC TTC AGG AGG GCC GGT GGC CTG TTC      5184
Ser Ala Ala Ser Glu Asp Asp Ile Phe Arg Arg Ala Gly Gly Leu Phe
        1715                1720                1725

GGC AAC CAC GTC AGC TAC TAC CAA AGC GAC GGC CGG AGC GCC TTC CCC      5232
Gly Asn His Val Ser Tyr Tyr Gln Ser Asp Gly Arg Ser Ala Phe Pro
    1730                1735                1740

CAG ACC TTC ACC ACT CAG CGC CCG CTG CAC ATC AAC AAG GCG GGC AGC      5280
Gln Thr Phe Thr Thr Gln Arg Pro Leu His Ile Asn Lys Ala Gly Ser
1745                1750                1755                1760

AGC CAG GGC GAC ACT GAG TCG CCA TCC CAC GAG AAG CTG GTG GAC TCC      5328
Ser Gln Gly Asp Thr Glu Ser Pro Ser His Glu Lys Leu Val Asp Ser
                1765                1770                1775

ACC TTC ACC CCG AGC AGC TAC TCG TCC ACC GGC TCC AAC GCC AAC ATC      5376
Thr Phe Thr Pro Ser Ser Tyr Ser Ser Thr Gly Ser Asn Ala Asn Ile
            1780                1785                1790

AAC AAC GCC AAC AAC ACC GCC CTG GGT CGC CTC CCT CGC CCC GCC GGC      5424
Asn Asn Ala Asn Asn Thr Ala Leu Gly Arg Leu Pro Arg Pro Ala Gly
        1795                1800                1805

TAC CCC AGC ACA GTC AGC ACT GTG GAG GGC CAC GGG CCC CCC TTG TCC      5472
Tyr Pro Ser Thr Val Ser Thr Val Glu Gly His Gly Pro Pro Leu Ser
    1810                1815                1820

CCT GCC ATC CGG GTG CAG GAG GTG GCG TGG AAG CTC AGC TCC AAC AGG      5520
Pro Ala Ile Arg Val Gln Glu Val Ala Trp Lys Leu Ser Ser Asn Arg
1825                1830                1835                1840

TGC CAC TCC CGG GAG AGC CAG GCA GCC ATG GCG CGT CAG GAG GAG ACG      5568
Cys His Ser Arg Glu Ser Gln Ala Ala Met Ala Arg Gln Glu Glu Thr
                1845                1850                1855

TCT CAG GAT GAG ACC TAT GAA GTG AAG ATG AAC CAT GAC ACG GAG GCC      5616
Ser Gln Asp Glu Thr Tyr Glu Val Lys Met Asn His Asp Thr Glu Ala
            1860                1865                1870

TGC AGT GAG CCC AGC CTG CTC TCC ACA GAG ATG CTC TCC TAC CAG GAT      5664
Cys Ser Glu Pro Ser Leu Leu Ser Thr Glu Met Leu Ser Tyr Gln Asp
        1875                1880                1885

GAC GAA AAT CGG CAA CTG ACG CTC CCA GAG GAG GAC AAG AGG GAC ATC      5712
Asp Glu Asn Arg Gln Leu Thr Leu Pro Glu Glu Asp Lys Arg Asp Ile
    1890                1895                1900

CGG CAA TCT CCG AAG AGG GGT TTC CTC CGC TCT TCC TCA CTA GGT CGA      5760
Arg Gln Ser Pro Lys Arg Gly Phe Leu Arg Ser Ser Ser Leu Gly Arg
1905                1910                1915                1920

AGG GCC TCC TTC CAC CTG GAA TGT CTG AAG CGA CAG AAG GAC CGA GGG      5808
Arg Ala Ser Phe His Leu Glu Cys Leu Lys Arg Gln Lys Asp Arg Gly
                1925                1930                1935

GGA GAC ATC TCT CAG AAG ACA GTC CTG CCC TTG CAT CTG GTT CAT CAT      5856
Gly Asp Ile Ser Gln Lys Thr Val Leu Pro Leu His Leu Val His His
            1940                1945                1950

CAG GCA TTG GCA GTG GCA GGC CTG AGC CCC CTC CTC CAG AGA AGC CAT      5904
Gln Ala Leu Ala Val Ala Gly Leu Ser Pro Leu Leu Gln Arg Ser His
        1955                1960                1965
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 132 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AGACCACGGC TTCCTCGAAT CTTGCGCGAA GCCGCCGGCC TCGGAGGAGG GATTAATCCA 60

GACCCGCCGG GGGGTGTTTT CACATTTCTT CCTCTTCGTG GCTGCTCCTC CTATTAAAAC 120

CATTTTTGGT CC 132

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 89 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGCTGAGGGC CTTCCGCGTG CTGCGCCCCC TGCGGCTGGT GTCCGGAGTC CCAAGTCTCC 60

AGGTGGTCCT GAATTCCATC ATCAAGGCC 89

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..84
(D) OTHER INFORMATION: /note= "An alternative exon of alpha-1C."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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CAC TAT TTC TGT GAT GCA TGG AAT ACA TTT GAC GCC TTG ATT GTT GTG        48
His Tyr Phe Cys Asp Ala Trp Asn Thr Phe Asp Ala Leu Ile Val Val
 1               5                  10                  15

GGT AGC ATT GTT GAT ATA GCA ATC ACC GAG GTA AAC                      84
Gly Ser Ile Val Asp Ile Ala Ile Thr Glu Val Asn
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7362 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 144..7163

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..143

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 7161..7362

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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GCGGCGGCGG CTGCGGCGGT GGGGCCGGGC GAGGTCCGTG CGGTCCCGGC GGCTCCGTGG      60

CTGCTCCGCT CTGAGCGCCT GCGCGCCCCG CGCCCTCCCT GCCGGGGCCG CTGGGCCGGG     120

GATGCACGCG GGGCCCGGGA GCC ATG GTC CGC TTC GGG GAC GAG CTG GGC          170
                         Met Val Arg Phe Gly Asp Glu Leu Gly
                           1               5

GGC CGC TAT GGA GGC CCC GGC GGC GGA GAG CGG GCC CGG GGC GGC GGG        218
Gly Arg Tyr Gly Gly Pro Gly Gly Gly Glu Arg Ala Arg Gly Gly Gly
 10                  15                  20                  25

GCC GGC GGG GCG GGG GGC CCG GGT CCC GGG GGG CTG CAG CCC GGC CAG       266
Ala Gly Gly Ala Gly Gly Pro Gly Pro Gly Gly Leu Gln Pro Gly Gln
                30                  35                  40

CGG GTC CTC TAC AAG CAA TCG ATC GCG CAG CGC GCG CGG ACC ATG GCG       314
Arg Val Leu Tyr Lys Gln Ser Ile Ala Gln Arg Ala Arg Thr Met Ala
            45                  50                  55

CTG TAC AAC CCC ATC CCG GTC AAG CAG AAC TGC TTC ACC GTC AAC CGC       362
Leu Tyr Asn Pro Ile Pro Val Lys Gln Asn Cys Phe Thr Val Asn Arg
        60                  65                  70

TCG CTC TTC GTC TTC AGC GAG GAC AAC GTC GTC CGC AAA TAC GCG AAG       410
Ser Leu Phe Val Phe Ser Glu Asp Asn Val Val Arg Lys Tyr Ala Lys
    75                  80                  85

CGC ATC ACC GAG TGG CCT CCA TTC GAG AAT ATG ATC CTG GCC ACC ATC       458
Arg Ile Thr Glu Trp Pro Pro Phe Glu Asn Met Ile Leu Ala Thr Ile
 90                  95                  100                 105

ATC GCC AAC TGC ATC GTG CTG GCC CTG GAG CAG CAC CTC CCT GAT GGG       506
Ile Ala Asn Cys Ile Val Leu Ala Leu Glu Gln His Leu Pro Asp Gly
                110                 115                 120

GAC AAA ACG CCC ATG TCC GAG CGG CTG GAC GAC ACG GAG CCC TAT TTC       554
Asp Lys Thr Pro Met Ser Glu Arg Leu Asp Asp Thr Glu Pro Tyr Phe
            125                 130                 135

ATC GGG ATC TTT TGC TTC GAG GCA GGG ATC AAA ATC ATC GCT CTG GGC       602
Ile Gly Ile Phe Cys Phe Glu Ala Gly Ile Lys Ile Ile Ala Leu Gly
        140                 145                 150

TTT GTC TTC CAC AAG GGC TCT TAC CTG CGG AAC GGC TGG AAC GTC ATG       650
Phe Val Phe His Lys Gly Ser Tyr Leu Arg Asn Gly Trp Asn Val Met
    155                 160                 165

GAC TTC GTG GTC GTC CTC ACA GGG ATC CTT GCC ACG GCT GGA ACT GAC       698
Asp Phe Val Val Val Leu Thr Gly Ile Leu Ala Thr Ala Gly Thr Asp
170                 175                 180                 185
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TTC GAC CTG CGA ACA CTG AGG GCT GTG CGT GTG CTG AGG CCC CTG AAG        746
Phe Asp Leu Arg Thr Leu Arg Ala Val Arg Val Leu Arg Pro Leu Lys
                190                 195                 200

CTG GTG TCT GGG ATT CCA AGT TTG CAG GTG GTG CTC AAG TCC ATC ATG        794
Leu Val Ser Gly Ile Pro Ser Leu Gln Val Val Leu Lys Ser Ile Met
            205                 210                 215

AAG GCC ATG GTT CCA CTC CTG CAG ATT GGG CTG CTT CTC TTC TTT GCC        842
Lys Ala Met Val Pro Leu Leu Gln Ile Gly Leu Leu Leu Phe Phe Ala
        220                 225                 230

ATC CTC ATG TTT GCC ATC ATT GGC CTG GAG TTC TAC ATG GGC AAG TTC        890
Ile Leu Met Phe Ala Ile Ile Gly Leu Glu Phe Tyr Met Gly Lys Phe
    235                 240                 245

CAC AAG GCC TGT TTC CCC AAC AGC ACA GAT GCG GAG CCC GTG GGT GAC        938
His Lys Ala Cys Phe Pro Asn Ser Thr Asp Ala Glu Pro Val Gly Asp
250                 255                 260                 265

TTC CCC TGT GGC AAG GAG GCC CCA GCC CGG CTG TGC GAG GGC GAC ACT        986
Phe Pro Cys Gly Lys Glu Ala Pro Ala Arg Leu Cys Glu Gly Asp Thr
                270                 275                 280

GAG TGC CGG GAG TAC TGG CCA GGA CCC AAC TTT GGC ATC ACC AAC TTT       1034
Glu Cys Arg Glu Tyr Trp Pro Gly Pro Asn Phe Gly Ile Thr Asn Phe
            285                 290                 295

GAC AAT ATC CTG TTT GCC ATC TTG ACG GTG TTC CAG TGC ATC ACC ATG       1082
Asp Asn Ile Leu Phe Ala Ile Leu Thr Val Phe Gln Cys Ile Thr Met
        300                 305                 310

GAG GGC TGG ACT GAC ATC CTC TAT AAT ACA AAC GAT GCG GCC GGC AAC       1130
Glu Gly Trp Thr Asp Ile Leu Tyr Asn Thr Asn Asp Ala Ala Gly Asn
    315                 320                 325

ACC TGG AAC TGG CTC TAC TTC ATC CCT CTC ATC ATC ATC GGC TCC TTC       1178
Thr Trp Asn Trp Leu Tyr Phe Ile Pro Leu Ile Ile Ile Gly Ser Phe
330                 335                 340                 345

TTC ATG CTC AAC CTG GTG CTG GGC GTG CTC TCG GGG GAG TTT GCC AAG       1226
Phe Met Leu Asn Leu Val Leu Gly Val Leu Ser Gly Glu Phe Ala Lys
                350                 355                 360

GAG CGA GAG AGG GTG GAG AAC CGC CGC GCC TTC CTG AAG CTG CGC CGG       1274
Glu Arg Glu Arg Val Glu Asn Arg Arg Ala Phe Leu Lys Leu Arg Arg
            365                 370                 375

CAG CAG CAG ATC GAG CGA GAG CTC AAC GGG TAC CTG GAG TGG ATC TTC       1322
Gln Gln Gln Ile Glu Arg Glu Leu Asn Gly Tyr Leu Glu Trp Ile Phe
        380                 385                 390

AAG GCG GAG GAA GTC ATG CTG GCC GAG GAG GAC AGG AAT GCA GAG GAG       1370
Lys Ala Glu Glu Val Met Leu Ala Glu Glu Asp Arg Asn Ala Glu Glu
    395                 400                 405

AAG TCC CCT TTG GAC GTG CTG AAG AGA GCG GCC ACC AAG AAG AGC AGA       1418
Lys Ser Pro Leu Asp Val Leu Lys Arg Ala Ala Thr Lys Lys Ser Arg
410                 415                 420                 425

AAT GAC CTG ATC CAC GCA GAG GAG GGA GAG GAC CGG TTT GCA GAT CTC       1466
Asn Asp Leu Ile His Ala Glu Glu Gly Glu Asp Arg Phe Ala Asp Leu
                430                 435                 440

TGT GCT GTT GGA TCC CCC TTC GCC CGC GCC AGC CTC AAG AGC GGG AAG       1514
Cys Ala Val Gly Ser Pro Phe Ala Arg Ala Ser Leu Lys Ser Gly Lys
            445                 450                 455
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ACA GAG AGC TCG TCA TAC TTC CGG AGG AAG GAG AAG ATG TTC CGG TTT      1562
Thr Glu Ser Ser Ser Tyr Phe Arg Arg Lys Glu Lys Met Phe Arg Phe
        460                 465                 470

TTT ATC CGG CGC ATG GTG AAG GCT CAG AGC TTC TAC TGG GTG GTG CTG      1610
Phe Ile Arg Arg Met Val Lys Ala Gln Ser Phe Tyr Trp Val Val Leu
    475                 480                 485

TGC GTG GTG GCC CTG AAC ACA CTG TGT GTG GCC ATG GTG CAT TAC AAC      1658
Cys Val Val Ala Leu Asn Thr Leu Cys Val Ala Met Val His Tyr Asn
490                 495                 500                 505

CAG CCG CGG CGG CTT ACC ACG ACC CTG TAT TTT GCA GAG TTT GTT TTC      1706
Gln Pro Arg Arg Leu Thr Thr Thr Leu Tyr Phe Ala Glu Phe Val Phe
                510                 515                 520

CTG GGT CTC TTC CTC ACA GAG ATG TCC CTG AAG ATG TAT GGC CTG GGG      1754
Leu Gly Leu Phe Leu Thr Glu Met Ser Leu Lys Met Tyr Gly Leu Gly
            525                 530                 535

CCC AGA AGC TAC TTC CGG TCC TCC TTC AAC TGC TTC GAC TTT GGG GTC      1802
Pro Arg Ser Tyr Phe Arg Ser Ser Phe Asn Cys Phe Asp Phe Gly Val
        540                 545                 550

ATC GTG GGG AGC GTC TTT GAA GTG GTC TGG GCG GCC ATC AAG CCG GGA      1850
Ile Val Gly Ser Val Phe Glu Val Val Trp Ala Ala Ile Lys Pro Gly
    555                 560                 565

AGC TCC TTT GGG ATC AGT GTG CTG CGG GCC CTC CGC CTG CTG AGG ATC      1898
Ser Ser Phe Gly Ile Ser Val Leu Arg Ala Leu Arg Leu Leu Arg Ile
570                 575                 580                 585

TTC AAA GTC ACG AAG TAC TGG AGC TCC CTG CGG AAC CTG GTG GTG TCC      1946
Phe Lys Val Thr Lys Tyr Trp Ser Ser Leu Arg Asn Leu Val Val Ser
                590                 595                 600

CTG CTG AAC TCC ATG AAG TCC ATC ATC AGC CTG CTC TTC TTG CTC TTC      1994
Leu Leu Asn Ser Met Lys Ser Ile Ile Ser Leu Leu Phe Leu Leu Phe
            605                 610                 615

CTG TTC ATT GTG GTC TTC GCC CTG CTG GGG ATG CAG CTG TTT GGG GGA      2042
Leu Phe Ile Val Val Phe Ala Leu Leu Gly Met Gln Leu Phe Gly Gly
        620                 625                 630

CAG TTC AAC TTC CAG GAT GAG ACT CCC ACA ACC AAC TTC GAC ACC TTC      2090
Gln Phe Asn Phe Gln Asp Glu Thr Pro Thr Thr Asn Phe Asp Thr Phe
    635                 640                 645

CCT GCC GCC ATC CTC ACT GTC TTC CAG ATC CTG ACG GGA GAG GAC TGG      2138
Pro Ala Ala Ile Leu Thr Val Phe Gln Ile Leu Thr Gly Glu Asp Trp
650                 655                 660                 665

AAT GCA GTG ATG TAT CAC GGG ATC GAA TCG CAA GGC GGC GTC AGC AAA      2186
Asn Ala Val Met Tyr His Gly Ile Glu Ser Gln Gly Gly Val Ser Lys
                670                 675                 680

GGC ATG TTC TCG TCC TTT TAC TTC ATT GTC CTG ACA CTG TTC GGA AAC      2234
Gly Met Phe Ser Ser Phe Tyr Phe Ile Val Leu Thr Leu Phe Gly Asn
            685                 690                 695

TAC ACT CTG CTG AAT GTC TTT CTG GCC ATC GCT GTG GAC AAC CTG GCC      2282
Tyr Thr Leu Leu Asn Val Phe Leu Ala Ile Ala Val Asp Asn Leu Ala
        700                 705                 710

AAC GCC CAA GAG CTG ACC AAG GAT GAA GAG GAG ATG GAA GAA GCA GCC      2330
Asn Ala Gln Glu Leu Thr Lys Asp Glu Glu Glu Met Glu Glu Ala Ala
    715                 720                 725
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AAT CAG AAG CTT GCT CTG CAA AAG GCC AAA GAA GTG GCT GAA GTC AGC      2378
Asn Gln Lys Leu Ala Leu Gln Lys Ala Lys Glu Val Ala Glu Val Ser
730                 735                 740                 745

CCC ATG TCT GCC GCG AAC ATC TCC ATC GCC GCC AGG CAG CAG AAC TCG      2426
Pro Met Ser Ala Ala Asn Ile Ser Ile Ala Ala Arg Gln Gln Asn Ser
                750                 755                 760

GCC AAG GCG CGC TCG GTG TGG GAG CAG CGG GCC AGC CAG CTA CGG CTG      2474
Ala Lys Ala Arg Ser Val Trp Glu Gln Arg Ala Ser Gln Leu Arg Leu
            765                 770                 775

CAG AAC CTG CGG GCC AGC TGC GAG GCG CTG TAC AGC GAG ATG GAC CCC      2522
Gln Asn Leu Arg Ala Ser Cys Glu Ala Leu Tyr Ser Glu Met Asp Pro
        780                 785                 790

GAG GAG CGG CTG CGC TTC GCC ACT ACG CGC CAC CTG CGG CCC GAC ATG      2570
Glu Glu Arg Leu Arg Phe Ala Thr Thr Arg His Leu Arg Pro Asp Met
    795                 800                 805

AAG ACG CAC CTG GAC CGG CCG CTG GTG GTG GAG CTG GGC CGC GAC GGC      2618
Lys Thr His Leu Asp Arg Pro Leu Val Val Glu Leu Gly Arg Asp Gly
810                 815                 820                 825

GCG CGG GGG CCC GTG GGA GGC AAA GCC CGA CCT GAG GCT GCG GAG GCC      2666
Ala Arg Gly Pro Val Gly Gly Lys Ala Arg Pro Glu Ala Ala Glu Ala
                830                 835                 840

CCC GAG GGC GTC GAC CCT CCG CGC AGG CAC CAC CGG CAC CGC GAC AAG      2714
Pro Glu Gly Val Asp Pro Pro Arg Arg His His Arg His Arg Asp Lys
            845                 850                 855

GAC AAG ACC CCC GCG GCG GGG GAC CAG GAC CGA GCA GAG GCC CCG AAG      2762
Asp Lys Thr Pro Ala Ala Gly Asp Gln Asp Arg Ala Glu Ala Pro Lys
        860                 865                 870

GCG GAG AGC GGG GAG CCC GGT GCC CGG GAG GAG CGG CCG CGG CCG CAC      2810
Ala Glu Ser Gly Glu Pro Gly Ala Arg Glu Glu Arg Pro Arg Pro His
    875                 880                 885

CGC AGC CAC AGC AAG GAG GCC GCG GGG CCC CCG GAG GCG CGG AGC GAG      2858
Arg Ser His Ser Lys Glu Ala Ala Gly Pro Pro Glu Ala Arg Ser Glu
890                 895                 900                 905

CGC GGC CGA GGC CCA GGC CCC GAG GGC GGC CGG CGG CAC CAC CGG CGC      2906
Arg Gly Arg Gly Pro Gly Pro Glu Gly Gly Arg Arg His His Arg Arg
                910                 915                 920

GGC TCC CCG GAG GAG GCG GCC GAG CGG GAG CCC CGA CGC CAC CGC GCG      2954
Gly Ser Pro Glu Glu Ala Ala Glu Arg Glu Pro Arg Arg His Arg Ala
            925                 930                 935

CAC CGG CAC CAG GAT CCG AGC AAG GAG TGC GCC GGC GCC AAG GGC GAG      3002
His Arg His Gln Asp Pro Ser Lys Glu Cys Ala Gly Ala Lys Gly Glu
        940                 945                 950

CGG CGC GCG CGG CAC CGC GGC GGC CCC CGA GCG GGG CCC CGG GAG GCG      3050
Arg Arg Ala Arg His Arg Gly Gly Pro Arg Ala Gly Pro Arg Glu Ala
    955                 960                 965

GAG AGC GGG GAG GAG CCG GCG CGG CGG CAC CGG GCC CGG CAC AAG GCG      3098
Glu Ser Gly Glu Glu Pro Ala Arg Arg His Arg Ala Arg His Lys Ala
970                 975                 980                 985

CAG CCT GCT CAC GAG GCT GTG GAG AAG GAG ACC ACG GAG AAG GAG GCC      3146
Gln Pro Ala His Glu Ala Val Glu Lys Glu Thr Thr Glu Lys Glu Ala
                990                 995                1000
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ACG GAG AAG GAG GCT GAG ATA GTG GAA GCC GAC AAG GAA AAG GAG CTC      3194
Thr Glu Lys Glu Ala Glu Ile Val Glu Ala Asp Lys Glu Lys Glu Leu
          1005                1010                1015

CGG AAC CAC CAG CCC CGG GAG CCA CAC TGT GAC CTG GAG ACC AGT GGG      3242
Arg Asn His Gln Pro Arg Glu Pro His Cys Asp Leu Glu Thr Ser Gly
     1020                1025                1030

ACT GTG ACT GTG GGT CCC ATG CAC ACA CTG CCC AGC ACC TGT CTC CAG      3290
Thr Val Thr Val Gly Pro Met His Thr Leu Pro Ser Thr Cys Leu Gln
1035                1040                1045

AAG GTG GAG GAA CAG CCA GAG GAT GCA GAC AAT CAG CGG AAC GTC ACT      3338
Lys Val Glu Glu Gln Pro Glu Asp Ala Asp Asn Gln Arg Asn Val Thr
1050                1055                1060                1065

CGC ATG GGC AGT CAG CCC CCA GAC CCG AAC ACT ATT GTA CAT ATC CCA      3386
Arg Met Gly Ser Gln Pro Pro Asp Pro Asn Thr Ile Val His Ile Pro
               1070                1075                1080

GTG ATG CTG ACG GGC CCT CTT GGG GAA GCC ACG GTC GTT CCC AGT GGT      3434
Val Met Leu Thr Gly Pro Leu Gly Glu Ala Thr Val Val Pro Ser Gly
          1085                1090                1095

AAC GTG GAC CTG GAA AGC CAA GCA GAG GGG AAG AAG GAG GTG GAA GCG      3482
Asn Val Asp Leu Glu Ser Gln Ala Glu Gly Lys Lys Glu Val Glu Ala
     1100                1105                1110

GAT GAC GTG ATG AGG AGC GGC CCC CGG CCT ATC GTC CCA TAC AGC TCC      3530
Asp Asp Val Met Arg Ser Gly Pro Arg Pro Ile Val Pro Tyr Ser Ser
1115                1120                1125

ATG TTC TGT TTA AGC CCC ACC AAC CTG CTC CGC CGC TTC TGC CAC TAC      3578
Met Phe Cys Leu Ser Pro Thr Asn Leu Leu Arg Arg Phe Cys His Tyr
1130                1135                1140                1145

ATC GTG ACC ATG AGG TAC TTC GAG GTG GTC ATT CTC GTG GTC ATC GCC      3626
Ile Val Thr Met Arg Tyr Phe Glu Val Val Ile Leu Val Val Ile Ala
               1150                1155                1160

TTG AGC AGC ATC GCC CTG GCT GCT GAG GAC CCA GTG CGC ACA GAC TCG      3674
Leu Ser Ser Ile Ala Leu Ala Ala Glu Asp Pro Val Arg Thr Asp Ser
          1165                1170                1175

CCC AGG AAC AAC GCT CTG AAA TAC CTG GAT TAC ATT TTC ACT GGT GTC      3722
Pro Arg Asn Asn Ala Leu Lys Tyr Leu Asp Tyr Ile Phe Thr Gly Val
     1180                1185                1190

TTT ACC TTT GAG ATG GTG ATA AAG ATG ATC GAC TTG GGA CTG CTG CTT      3770
Phe Thr Phe Glu Met Val Ile Lys Met Ile Asp Leu Gly Leu Leu Leu
1195                1200                1205

CAC CCT GGA GCC TAT TTC CGG GAC TTG TGG AAC ATT CTG GAC TTC ATT      3818
His Pro Gly Ala Tyr Phe Arg Asp Leu Trp Asn Ile Leu Asp Phe Ile
1210                1215                1220                1225

GTG GTC AGT GGC GCC CTG GTG GCG TTT GCT TTC TCA GGA TCC AAA GGG      3866
Val Val Ser Gly Ala Leu Val Ala Phe Ala Phe Ser Gly Ser Lys Gly
               1230                1235                1240

AAA GAC ATC AAT ACC ATC AAG TCT CTG AGA GTC CTT CGT GTC CTG CGG      3914
Lys Asp Ile Asn Thr Ile Lys Ser Leu Arg Val Leu Arg Val Leu Arg
          1245                1250                1255

CCC CTC AAG ACC ATC AAA CGG CTG CCC AAG CTC AAG GCT GTG TTT GAC      3962
Pro Leu Lys Thr Ile Lys Arg Leu Pro Lys Leu Lys Ala Val Phe Asp
     1260                1265                1270
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TGT GTG GTG AAC TCC CTG AAG AAT GTC CTC AAC ATC TTG ATT GTC TAC      4010
Cys Val Val Asn Ser Leu Lys Asn Val Leu Asn Ile Leu Ile Val Tyr
    1275                1280                1285

ATG CTC TTC ATG TTC ATA TTT GCC GTC ATT GCG GTG CAG CTC TTC AAA      4058
Met Leu Phe Met Phe Ile Phe Ala Val Ile Ala Val Gln Leu Phe Lys
1290                1295                1300                1305

GGG AAG TTT TTC TAC TGC ACA GAT GAA TCC AAG GAG CTG GAG AGG GAC      4106
Gly Lys Phe Phe Tyr Cys Thr Asp Glu Ser Lys Glu Leu Glu Arg Asp
                1310                1315                1320

TGC AGG GGT CAG TAT TTG GAT TAT GAG AAG GAG GAA GTG GAA GCT CAG      4154
Cys Arg Gly Gln Tyr Leu Asp Tyr Glu Lys Glu Glu Val Glu Ala Gln
            1325                1330                1335

CCC AGG CAG TGG AAG AAA TAC GAC TTT CAC TAC GAC AAT GTG CTC TGG      4202
Pro Arg Gln Trp Lys Lys Tyr Asp Phe His Tyr Asp Asn Val Leu Trp
        1340                1345                1350

GCT CTG CTG ACG CTG TTC ACA GTG TCC ACG GGA GAA GGC TGG CCC ATG      4250
Ala Leu Leu Thr Leu Phe Thr Val Ser Thr Gly Glu Gly Trp Pro Met
    1355                1360                1365

GTG CTG AAA CAC TCC GTG GAT GCC ACC TAT GAG GAG CAG GGT CCA AGC      4298
Val Leu Lys His Ser Val Asp Ala Thr Tyr Glu Glu Gln Gly Pro Ser
1370                1375                1380                1385

CCT GGG TAC CGC ATG GAG CTG TCC ATC TTC TAC GTG GTC TAC TTT GTG      4346
Pro Gly Tyr Arg Met Glu Leu Ser Ile Phe Tyr Val Val Tyr Phe Val
                1390                1395                1400

GTC TTT CCC TTC TTC TTC GTC AAC ATC TTT GTG GCT TTG ATC ATC ATC      4394
Val Phe Pro Phe Phe Phe Val Asn Ile Phe Val Ala Leu Ile Ile Ile
            1405                1410                1415

ACC TTC CAG GAG CAG GGG GAC AAG GTG ATG TCT GAA TGC AGC CTG GAG      4442
Thr Phe Gln Glu Gln Gly Asp Lys Val Met Ser Glu Cys Ser Leu Glu
        1420                1425                1430

AAG AAC GAG AGG GCT TGC ATT GAC TTC GCC ATC AGC GCC AAA CCC CTG      4490
Lys Asn Glu Arg Ala Cys Ile Asp Phe Ala Ile Ser Ala Lys Pro Leu
    1435                1440                1445

ACA CGG TAC ATG CCC CAA AAC CGG CAG TCG TTC CAG TAT AAG ACG TGG      4538
Thr Arg Tyr Met Pro Gln Asn Arg Gln Ser Phe Gln Tyr Lys Thr Trp
1450                1455                1460                1465

ACA TTT GTG GTC TCC CCG CCC TTT GAA TAC TTC ATC ATG GCC ATG ATA      4586
Thr Phe Val Val Ser Pro Pro Phe Glu Tyr Phe Ile Met Ala Met Ile
                1470                1475                1480

GCC CTC AAC ACT GTG GTG CTG ATG ATG AAG TTC TAT GAT GCA CCC TAT      4634
Ala Leu Asn Thr Val Val Leu Met Met Lys Phe Tyr Asp Ala Pro Tyr
            1485                1490                1495

GAG TAC GAG CTG ATG CTG AAA TGC CTG AAC ATC GTG TTC ACA TCC ATG      4682
Glu Tyr Glu Leu Met Leu Lys Cys Leu Asn Ile Val Phe Thr Ser Met
        1500                1505                1510

TTC TCC ATG GAA TGC GTG CTG AAG ATC ATC GCC TTT GGG GTG CTG AAC      4730
Phe Ser Met Glu Cys Val Leu Lys Ile Ile Ala Phe Gly Val Leu Asn
    1515                1520                1525

TAT TTC AGA GAT GCC TGG AAT GTC TTT GAC TTT GTC ACT GTG TTG GGA      4778
Tyr Phe Arg Asp Ala Trp Asn Val Phe Asp Phe Val Thr Val Leu Gly
1530                1535                1540                1545
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AGT ATT ACT GAT ATT TTA GTA ACA GAG ATT GCG GAA ACG AAC AAT TTC      4826
Ser Ile Thr Asp Ile Leu Val Thr Glu Ile Ala Glu Thr Asn Asn Phe
                1550                1555                1560

ATC AAC CTC AGC TTC CTC CGC CTC TTT CGA GCT GCG CGG CTG ATC AAG      4874
Ile Asn Leu Ser Phe Leu Arg Leu Phe Arg Ala Ala Arg Leu Ile Lys
            1565                1570                1575

CTG CTC CGC CAG GGC TAC ACC ATC CGC ATC CTG CTG TGG ACC TTT GTC      4922
Leu Leu Arg Gln Gly Tyr Thr Ile Arg Ile Leu Leu Trp Thr Phe Val
        1580                1585                1590

CAG TCC TTC AAG GCC CTG CCC TAC GTG TGT CTG CTC ATT GCC ATG CTG      4970
Gln Ser Phe Lys Ala Leu Pro Tyr Val Cys Leu Leu Ile Ala Met Leu
    1595                1600                1605

TTC TTC ATC TAC GCC ATC ATC GGC ATG CAG GTG TTT GGG AAT ATT GCC      5018
Phe Phe Ile Tyr Ala Ile Ile Gly Met Gln Val Phe Gly Asn Ile Ala
1610                1615                1620                1625

CTG GAT GAT GAC ACC AGC ATC AAC CGC CAC AAC AAC TTC CGG ACG TTT      5066
Leu Asp Asp Asp Thr Ser Ile Asn Arg His Asn Asn Phe Arg Thr Phe
                1630                1635                1640

TTG CAA GCC CTG ATG CTG CTG TTC AGG AGC GCC ACG GGG GAG GCC TGG      5114
Leu Gln Ala Leu Met Leu Leu Phe Arg Ser Ala Thr Gly Glu Ala Trp
            1645                1650                1655

CAC GAG ATC ATG CTG TCC TGC CTG AGC AAC CAG GCC TGT GAT GAG CAG      5162
His Glu Ile Met Leu Ser Cys Leu Ser Asn Gln Ala Cys Asp Glu Gln
        1660                1665                1670

GCC AAT GCC ACC GAG TGT GGA AGT GAC TTT GCC TAC TTC TAC TTC GTC      5210
Ala Asn Ala Thr Glu Cys Gly Ser Asp Phe Ala Tyr Phe Tyr Phe Val
    1675                1680                1685

TCC TTC ATC TTC CTG TGC TCC TTT CTG ATG TTG AAC CTC TTT GTG GCT      5258
Ser Phe Ile Phe Leu Cys Ser Phe Leu Met Leu Asn Leu Phe Val Ala
1690                1695                1700                1705

GTG ATC ATG GAC AAT TTT GAG TAC CTC ACG CGG GAC TCT TCC ATC CTA      5306
Val Ile Met Asp Asn Phe Glu Tyr Leu Thr Arg Asp Ser Ser Ile Leu
                1710                1715                1720

GGT CCT CAC CAC TTG GAT GAG TTC ATC CGG GTC TGG GCT GAA TAC GAC      5354
Gly Pro His His Leu Asp Glu Phe Ile Arg Val Trp Ala Glu Tyr Asp
            1725                1730                1735

CCG GCT GCG TGT GGG CGC ATC AGT TAC AAT GAC ATG TTT GAG ATG CTG      5402
Pro Ala Ala Cys Gly Arg Ile Ser Tyr Asn Asp Met Phe Glu Met Leu
        1740                1745                1750

AAA CAC ATG TCC CCG CCT CTG GGG CTG GGG AAG AAA TGC CCT GCT CGA      5450
Lys His Met Ser Pro Pro Leu Gly Leu Gly Lys Lys Cys Pro Ala Arg
    1755                1760                1765

GTT GCT TAC AAG CGC CTG GTT CGC ATG AAC ATG CCC ATC TCC AAC GAG      5498
Val Ala Tyr Lys Arg Leu Val Arg Met Asn Met Pro Ile Ser Asn Glu
1770                1775                1780                1785

GAC ATG ACT GTT CAC TTC ACG TCC ACG CTG ATG GCC CTC ATC CGG ACG      5546
Asp Met Thr Val His Phe Thr Ser Thr Leu Met Ala Leu Ile Arg Thr
                1790                1795                1800

GCA CTG GAG ATC AAG CTG GCC CCA GCT GGG ACA AAG CAG CAT CAG TGT      5594
Ala Leu Glu Ile Lys Leu Ala Pro Ala Gly Thr Lys Gln His Gln Cys
            1805                1810                1815
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CCC CCG GGA GAG GGG CCT ACA GGC TGC CGG CGG GAA CGA GAG CGC CGG      6458
Pro Pro Gly Glu Gly Pro Thr Gly Cys Arg Arg Glu Arg Glu Arg Arg
2090                2095                2100                2105

CAG GAG CGG GGC CGG TCC CAG GAG CGG AGG CAG CCC TCA TCC TCC TCC      6506
Gln Glu Arg Gly Arg Ser Gln Glu Arg Arg Gln Pro Ser Ser Ser Ser
                    2110                2115                2120

TCG GAG AAG CAG CGC TTC TAC TCC TGC GAC CGC TTT GGG GGC CGT GAG      6554
Ser Glu Lys Gln Arg Phe Tyr Ser Cys Asp Arg Phe Gly Gly Arg Glu
            2125                2130                2135

CCC CCG AAG CCC AAG CCC TCC CTC AGC AGC CAC CCA ACG TCG CCA ACA      6602
Pro Pro Lys Pro Lys Pro Ser Leu Ser Ser His Pro Thr Ser Pro Thr
        2140                2145                2150

GCT GGC CAG GAG CCG GGA CCC CAC CCA CAG GGC AGT GGT TCC GTG AAT      6650
Ala Gly Gln Glu Pro Gly Pro His Pro Gln Gly Ser Gly Ser Val Asn
    2155                2160                2165

GGG AGC CCC TTG CTG TCA ACA TCT GGT GCT AGC ACC CCC GGC CGC GGT      6698
Gly Ser Pro Leu Leu Ser Thr Ser Gly Ala Ser Thr Pro Gly Arg Gly
2170                2175                2180                2185

GGG CGG AGG CAG CTC CCC CAG ACG CCC CTG ACT CCC CGC CCC AGC ATC      6746
Gly Arg Arg Gln Leu Pro Gln Thr Pro Leu Thr Pro Arg Pro Ser Ile
                    2190                2195                2200

ACC TAC AAG ACG GCC AAC TCC TCA CCC ATC CAC TTC GCC GGG GCT CAG      6794
Thr Tyr Lys Thr Ala Asn Ser Ser Pro Ile His Phe Ala Gly Ala Gln
            2205                2210                2215

ACC AGC CTC CCT GCC TTC TCC CCA GGC CGG CTC AGC CGT GGG CTT TCC      6842
Thr Ser Leu Pro Ala Phe Ser Pro Gly Arg Leu Ser Arg Gly Leu Ser
        2220                2225                2230

GAA CAC AAC GCC CTG CTG CAG AGA GAC CCC CTC AGC CAG CCC CTG GCC      6890
Glu His Asn Ala Leu Leu Gln Arg Asp Pro Leu Ser Gln Pro Leu Ala
    2235                2240                2245

CCT GGC TCT CGA ATT GGC TCT GAC CCT TAC CTG GGG CAG CGT CTG GAC      6938
Pro Gly Ser Arg Ile Gly Ser Asp Pro Tyr Leu Gly Gln Arg Leu Asp
2250                2255                2260                2265

AGT GAG GCC TCT GTC CAC GCC CTG CCT GAG GAC ACG CTC ACT TTC GAG      6986
Ser Glu Ala Ser Val His Ala Leu Pro Glu Asp Thr Leu Thr Phe Glu
                    2270                2275                2280

GAG GCT GTG GCC ACC AAC TCG GGC CGC TCC TCC AGG ACT TCC TAC GTG      7034
Glu Ala Val Ala Thr Asn Ser Gly Arg Ser Ser Arg Thr Ser Tyr Val
            2285                2290                2295

TCC TCC CTG ACC TCC CAG TCT CAC CCT CTC CGC CGC GTG CCC AAC GGT      7082
Ser Ser Leu Thr Ser Gln Ser His Pro Leu Arg Arg Val Pro Asn Gly
        2300                2305                2310

TAC CAC TGC ACC CTG GGA CTC AGC TCG GGT GGC CGA GCA CGG CAC AGC      7130
Tyr His Cys Thr Leu Gly Leu Ser Ser Gly Gly Arg Ala Arg His Ser
    2315                2320                2325

TAC CAC CAC CCT GAC CAA GAC CAC TGG TGC TAGCTGCACC GTGACCGCTC        7180
Tyr His His Pro Asp Gln Asp His Trp Cys
2330                2335                234

AGACGCCTGC ATGCAGCAGG CGTGTGTTCC AGTGGATGAG TTTTATCATC CACACGGGGC   7240

AGTCGGCCCT CGGGGGAGGC CTTGCCCACC TTGGTGAGGC TCCTGTGGCC CCTCCCTCCC   7300
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CCTCCTCCCC TCTTTTACTC TAGACGACGA ATAAAGCCCT GTTGCTTGAG TGTACGTACC    7360

GC                                                                   7362
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(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7175 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 144..6857

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..143

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 6855..7175

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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GCGGCGGCGG CTGCGGCGGT GGGGCCGGGC GAGGTCCGTG CGGTCCCGGC GGCTCCGTGG     60

CTGCTCCGCT CTGAGCGCCT GCGCGCCCCG CGCCCTCCCT GCCGGGGCCG CTGGGCCGGG    120

GATGCACGCG GGGCCCGGGA GCC ATG GTC CGC TTC GGG GAC GAG CTG GGC         170
                         Met Val Arg Phe Gly Asp Glu Leu Gly
                           1               5

GGC CGC TAT GGA GGC CCC GGC GGC GGA GAG CGG GCC CGG GGC GGC GGG       218
Gly Arg Tyr Gly Gly Pro Gly Gly Gly Glu Arg Ala Arg Gly Gly Gly
 10                  15                  20                  25

GCC GGC GGG GCG GGG GGC CCG GGT CCC GGG GGG CTG CAG CCC GGC CAG      266
Ala Gly Gly Ala Gly Gly Pro Gly Pro Gly Gly Leu Gln Pro Gly Gln
                 30                  35                  40

CGG GTC CTC TAC AAG CAA TCG ATC GCG CAG CGC GCG CGG ACC ATG GCG      314
Arg Val Leu Tyr Lys Gln Ser Ile Ala Gln Arg Ala Arg Thr Met Ala
             45                  50                  55

CTG TAC AAC CCC ATC CCG GTC AAG CAG AAC TGC TTC ACC GTC AAC CGC      362
Leu Tyr Asn Pro Ile Pro Val Lys Gln Asn Cys Phe Thr Val Asn Arg
         60                  65                  70

TCG CTC TTC GTC TTC AGC GAG GAC AAC GTC GTC CGC AAA TAC GCG AAG      410
Ser Leu Phe Val Phe Ser Glu Asp Asn Val Val Arg Lys Tyr Ala Lys
     75                  80                  85

CGC ATC ACC GAG TGG CCT CCA TTC GAG AAT ATG ATC CTG GCC ACC ATC      458
Arg Ile Thr Glu Trp Pro Pro Phe Glu Asn Met Ile Leu Ala Thr Ile
 90                  95                  100                 105

ATC GCC AAC TGC ATC GTG CTG GCC CTG GAG CAG CAC CTC CCT GAT GGG      506
Ile Ala Asn Cys Ile Val Leu Ala Leu Glu Gln His Leu Pro Asp Gly
                110                 115                 120

GAC AAA ACG CCC ATG TCC GAG CGG CTG GAC GAC ACG GAG CCC TAT TTC      554
Asp Lys Thr Pro Met Ser Glu Arg Leu Asp Asp Thr Glu Pro Tyr Phe
            125                 130                 135
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ATC GGG ATC TTT TGC TTC GAG GCA GGG ATC AAA ATC ATC GCT CTG GGC          602
Ile Gly Ile Phe Cys Phe Glu Ala Gly Ile Lys Ile Ile Ala Leu Gly
        140                 145                 150

TTT GTC TTC CAC AAG GGC TCT TAC CTG CGG AAC GGC TGG AAC GTC ATG          650
Phe Val Phe His Lys Gly Ser Tyr Leu Arg Asn Gly Trp Asn Val Met
    155                 160                 165

GAC TTC GTG GTC GTC CTC ACA GGG ATC CTT GCC ACG GCT GGA ACT GAC          698
Asp Phe Val Val Val Leu Thr Gly Ile Leu Ala Thr Ala Gly Thr Asp
170                 175                 180                 185

TTC GAC CTG CGA ACA CTG AGG GCT GTG CGT GTG CTG AGG CCC CTG AAG          746
Phe Asp Leu Arg Thr Leu Arg Ala Val Arg Val Leu Arg Pro Leu Lys
                190                 195                 200

CTG GTG TCT GGG ATT CCA AGT TTG CAG GTG GTG CTC AAG TCC ATC ATG          794
Leu Val Ser Gly Ile Pro Ser Leu Gln Val Val Leu Lys Ser Ile Met
            205                 210                 215

AAG GCC ATG GTT CCA CTC CTG CAG ATT GGG CTG CTT CTC TTC TTT GCC          842
Lys Ala Met Val Pro Leu Leu Gln Ile Gly Leu Leu Leu Phe Phe Ala
        220                 225                 230

ATC CTC ATG TTT GCC ATC ATT GGC CTG GAG TTC TAC ATG GGC AAG TTC          890
Ile Leu Met Phe Ala Ile Ile Gly Leu Glu Phe Tyr Met Gly Lys Phe
    235                 240                 245

CAC AAG GCC TGT TTC CCC AAC AGC ACA GAT GCG GAG CCC GTG GGT GAC          938
His Lys Ala Cys Phe Pro Asn Ser Thr Asp Ala Glu Pro Val Gly Asp
250                 255                 260                 265

TTC CCC TGT GGC AAG GAG GCC CCA GCC CGG CTG TGC GAG GGC GAC ACT          986
Phe Pro Cys Gly Lys Glu Ala Pro Ala Arg Leu Cys Glu Gly Asp Thr
                270                 275                 280

GAG TGC CGG GAG TAC TGG CCA GGA CCC AAC TTT GGC ATC ACC AAC TTT         1034
Glu Cys Arg Glu Tyr Trp Pro Gly Pro Asn Phe Gly Ile Thr Asn Phe
            285                 290                 295

GAC AAT ATC CTG TTT GCC ATC TTG ACG GTG TTC CAG TGC ATC ACC ATG         1082
Asp Asn Ile Leu Phe Ala Ile Leu Thr Val Phe Gln Cys Ile Thr Met
        300                 305                 310

GAG GGC TGG ACT GAC ATC CTC TAT AAT ACA AAC GAT GCG GCC GGC AAC         1130
Glu Gly Trp Thr Asp Ile Leu Tyr Asn Thr Asn Asp Ala Ala Gly Asn
    315                 320                 325

ACC TGG AAC TGG CTC TAC TTC ATC CCT CTC ATC ATC ATC GGC TCC TTC         1178
Thr Trp Asn Trp Leu Tyr Phe Ile Pro Leu Ile Ile Ile Gly Ser Phe
330                 335                 340                 345

TTC ATG CTC AAC CTG GTG CTG GGC GTG CTC TCG GGG GAG TTT GCC AAG         1226
Phe Met Leu Asn Leu Val Leu Gly Val Leu Ser Gly Glu Phe Ala Lys
                350                 355                 360

GAG CGA GAG AGG GTG GAG AAC CGC CGC GCC TTC CTG AAG CTG CGC CGG         1274
Glu Arg Glu Arg Val Glu Asn Arg Arg Ala Phe Leu Lys Leu Arg Arg
            365                 370                 375

CAG CAG CAG ATC GAG CGA GAG CTC AAC GGG TAC CTG GAG TGG ATC TTC         1322
Gln Gln Gln Ile Glu Arg Glu Leu Asn Gly Tyr Leu Glu Trp Ile Phe
        380                 385                 390

AAG GCG GAG GAA GTC ATG CTG GCC GAG GAG GAC AGG AAT GCA GAG GAG         1370
Lys Ala Glu Glu Val Met Leu Ala Glu Glu Asp Arg Asn Ala Glu Glu
    395                 400                 405
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AAG TCC CCT TTG GAC GTG CTG AAG AGA GCG GCC ACC AAG AAG AGC AGA      1418
Lys Ser Pro Leu Asp Val Leu Lys Arg Ala Ala Thr Lys Lys Ser Arg
410                 415                 420                 425

AAT GAC CTG ATC CAC GCA GAG GAG GGA GAG GAC CGG TTT GCA GAT CTC      1466
Asn Asp Leu Ile His Ala Glu Glu Gly Glu Asp Arg Phe Ala Asp Leu
                430                 435                 440

TGT GCT GTT GGA TCC CCC TTC GCC CGC GCC AGC CTC AAG AGC GGG AAG      1514
Cys Ala Val Gly Ser Pro Phe Ala Arg Ala Ser Leu Lys Ser Gly Lys
            445                 450                 455

ACA GAG AGC TCG TCA TAC TTC CGG AGG AAG GAG AAG ATG TTC CGG TTT      1562
Thr Glu Ser Ser Ser Tyr Phe Arg Arg Lys Glu Lys Met Phe Arg Phe
        460                 465                 470

TTT ATC CGG CGC ATG GTG AAG GCT CAG AGC TTC TAC TGG GTG GTG CTG      1610
Phe Ile Arg Arg Met Val Lys Ala Gln Ser Phe Tyr Trp Val Val Leu
    475                 480                 485

TGC GTG GTG GCC CTG AAC ACA CTG TGT GTG GCC ATG GTG CAT TAC AAC      1658
Cys Val Val Ala Leu Asn Thr Leu Cys Val Ala Met Val His Tyr Asn
490                 495                 500                 505

CAG CCG CGG CGG CTT ACC ACG ACC CTG TAT TTT GCA GAG TTT GTT TTC      1706
Gln Pro Arg Arg Leu Thr Thr Thr Leu Tyr Phe Ala Glu Phe Val Phe
                510                 515                 520

CTG GGT CTC TTC CTC ACA GAG ATG TCC CTG AAG ATG TAT GGC CTG GGG      1754
Leu Gly Leu Phe Leu Thr Glu Met Ser Leu Lys Met Tyr Gly Leu Gly
            525                 530                 535

CCC AGA AGC TAC TTC CGG TCC TCC TTC AAC TGC TTC GAC TTT GGG GTC      1802
Pro Arg Ser Tyr Phe Arg Ser Ser Phe Asn Cys Phe Asp Phe Gly Val
        540                 545                 550

ATC GTG GGG AGC GTC TTT GAA GTG GTC TGG GCG GCC ATC AAG CCG GGA      1850
Ile Val Gly Ser Val Phe Glu Val Val Trp Ala Ala Ile Lys Pro Gly
    555                 560                 565

AGC TCC TTT GGG ATC AGT GTG CTG CGG GCC CTC CGC CTG CTG AGG ATC      1898
Ser Ser Phe Gly Ile Ser Val Leu Arg Ala Leu Arg Leu Leu Arg Ile
570                 575                 580                 585

TTC AAA GTC ACG AAG TAC TGG AGC TCC CTG CGG AAC CTG GTG GTG TCC      1946
Phe Lys Val Thr Lys Tyr Trp Ser Ser Leu Arg Asn Leu Val Val Ser
                590                 595                 600

CTG CTG AAC TCC ATG AAG TCC ATC ATC AGC CTG CTC TTC TTG CTC TTC      1994
Leu Leu Asn Ser Met Lys Ser Ile Ile Ser Leu Leu Phe Leu Leu Phe
            605                 610                 615

CTG TTC ATT GTG GTC TTC GCC CTG CTG GGG ATG CAG CTG TTT GGG GGA      2042
Leu Phe Ile Val Val Phe Ala Leu Leu Gly Met Gln Leu Phe Gly Gly
        620                 625                 630

CAG TTC AAC TTC CAG GAT GAG ACT CCC ACA ACC AAC TTC GAC ACC TTC      2090
Gln Phe Asn Phe Gln Asp Glu Thr Pro Thr Thr Asn Phe Asp Thr Phe
    635                 640                 645

CCT GCC GCC ATC CTC ACT GTC TTC CAG ATC CTG ACG GGA GAG GAC TGG      2138
Pro Ala Ala Ile Leu Thr Val Phe Gln Ile Leu Thr Gly Glu Asp Trp
650                 655                 660                 665

AAT GCA GTG ATG TAT CAC GGG ATC GAA TCG CAA GGC GGC GTC AGC AAA      2186
Asn Ala Val Met Tyr His Gly Ile Glu Ser Gln Gly Gly Val Ser Lys
                670                 675                 680
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GGC ATG TTC TCG TCC TTT TAC TTC ATT GTC CTG ACA CTG TTC GGA AAC 2234
Gly Met Phe Ser Ser Phe Tyr Phe Ile Val Leu Thr Leu Phe Gly Asn
685 690 695

TAC ACT CTG CTG AAT GTC TTT CTG GCC ATC GCT GTG GAC AAC CTG GCC 2282
Tyr Thr Leu Leu Asn Val Phe Leu Ala Ile Ala Val Asp Asn Leu Ala
700 705 710

AAC GCC CAA GAG CTG ACC AAG GAT GAA GAG GAG ATG GAA GAA GCA GCC 2330
Asn Ala Gln Glu Leu Thr Lys Asp Glu Glu Glu Met Glu Glu Ala Ala
715 720 725

AAT CAG AAG CTT GCT CTG CAA AAG GCC AAA GAA GTG GCT GAA GTC AGC 2378
Asn Gln Lys Leu Ala Leu Gln Lys Ala Lys Glu Val Ala Glu Val Ser
730 735 740 745

CCC ATG TCT GCC GCG AAC ATC TCC ATC GCC GCC AGG CAG CAG AAC TCG 2426
Pro Met Ser Ala Ala Asn Ile Ser Ile Ala Ala Arg Gln Gln Asn Ser
750 755 760

GCC AAG GCG CGC TCG GTG TGG GAG CAG CGG GCC AGC CAG CTA CGG CTG 2474
Ala Lys Ala Arg Ser Val Trp Glu Gln Arg Ala Ser Gln Leu Arg Leu
765 770 775

CAG AAC CTG CGG GCC AGC TGC GAG GCG CTG TAC AGC GAG ATG GAC CCC 2522
Gln Asn Leu Arg Ala Ser Cys Glu Ala Leu Tyr Ser Glu Met Asp Pro
780 785 790

GAG GAG CGG CTG CGC TTC GCC ACT ACG CGC CAC CTG CGG CCC GAC ATG 2570
Glu Glu Arg Leu Arg Phe Ala Thr Thr Arg His Leu Arg Pro Asp Met
795 800 805

AAG ACG CAC CTG GAC CGG CCG CTG GTG GTG GAG CTG GGC CGC GAC GGC 2618
Lys Thr His Leu Asp Arg Pro Leu Val Val Glu Leu Gly Arg Asp Gly
810 815 820 825

GCG CGG GGG CCC GTG GGA GGC AAA GCC CGA CCT GAG GCT GCG GAG GCC 2666
Ala Arg Gly Pro Val Gly Gly Lys Ala Arg Pro Glu Ala Ala Glu Ala
830 835 840

CCC GAG GGC GTC GAC CCT CCG CGC AGG CAC CAC CGG CAC CGC GAC AAG 2714
Pro Glu Gly Val Asp Pro Pro Arg Arg His His Arg His Arg Asp Lys
845 850 855

GAC AAG ACC CCC GCG GCG GGG GAC CAG GAC CGA GCA GAG GCC CCG AAG 2762
Asp Lys Thr Pro Ala Ala Gly Asp Gln Asp Arg Ala Glu Ala Pro Lys
860 865 870

GCG GAG AGC GGG GAG CCC GGT GCC CGG GAG GAG CGG CCG CGG CCG CAC 2810
Ala Glu Ser Gly Glu Pro Gly Ala Arg Glu Glu Arg Pro Arg Pro His
875 880 885

CGC AGC CAC AGC AAG GAG GCC GCG GGG CCC CCG GAG GCG CGG AGC GAG 2858
Arg Ser His Ser Lys Glu Ala Ala Gly Pro Pro Glu Ala Arg Ser Glu
890 895 900 905

CGC GGC CGA GGC CCA GGC CCC GAG GGC GGC CGG CGG CAC CAC CGG CGC 2906
Arg Gly Arg Gly Pro Gly Pro Glu Gly Gly Arg Arg His His Arg Arg
910 915 920

GGC TCC CCG GAG GAG GCG GCC GAG CGG GAG CCC CGA CGC CAC CGC GCG 2954
Gly Ser Pro Glu Glu Ala Ala Glu Arg Glu Pro Arg Arg His Arg Ala
925 930 935

CAC CGG CAC CAG GAT CCG AGC AAG GAG TGC GCC GGC GCC AAG GGC GAG 3002
His Arg His Gln Asp Pro Ser Lys Glu Cys Ala Gly Ala Lys Gly Glu
940 945 950

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CGG CGC GCG CGG CAC CGC GGC GGC CCC CGA GCG GGG CCC CGG GAG GCG      3050
Arg Arg Ala Arg His Arg Gly Gly Pro Arg Ala Gly Pro Arg Glu Ala
    955                 960                 965

GAG AGC GGG GAG GAG CCG GCG CGG CGG CAC CGG GCC CGG CAC AAG GCG      3098
Glu Ser Gly Glu Glu Pro Ala Arg Arg His Arg Ala Arg His Lys Ala
970                 975                 980                 985

CAG CCT GCT CAC GAG GCT GTG GAG AAG GAG ACC ACG GAG AAG GAG GCC      3146
Gln Pro Ala His Glu Ala Val Glu Lys Glu Thr Thr Glu Lys Glu Ala
                990                 995                1000

ACG GAG AAG GAG GCT GAG ATA GTG GAA GCC GAC AAG GAA AAG GAG CTC      3194
Thr Glu Lys Glu Ala Glu Ile Val Glu Ala Asp Lys Glu Lys Glu Leu
            1005                1010                1015

CGG AAC CAC CAG CCC CGG GAG CCA CAC TGT GAC CTG GAG ACC AGT GGG      3242
Arg Asn His Gln Pro Arg Glu Pro His Cys Asp Leu Glu Thr Ser Gly
        1020                1025                1030

ACT GTG ACT GTG GGT CCC ATG CAC ACA CTG CCC AGC ACC TGT CTC CAG      3290
Thr Val Thr Val Gly Pro Met His Thr Leu Pro Ser Thr Cys Leu Gln
    1035                1040                1045

AAG GTG GAG GAA CAG CCA GAG GAT GCA GAC AAT CAG CGG AAC GTC ACT      3338
Lys Val Glu Glu Gln Pro Glu Asp Ala Asp Asn Gln Arg Asn Val Thr
1050                1055                1060                1065

CGC ATG GGC AGT CAG CCC CCA GAC CCG AAC ACT ATT GTA CAT ATC CCA      3386
Arg Met Gly Ser Gln Pro Pro Asp Pro Asn Thr Ile Val His Ile Pro
                1070                1075                1080

GTG ATG CTG ACG GGC CCT CTT GGG GAA GCC ACG GTC GTT CCC AGT GGT      3434
Val Met Leu Thr Gly Pro Leu Gly Glu Ala Thr Val Val Pro Ser Gly
            1085                1090                1095

AAC GTG GAC CTG GAA AGC CAA GCA GAG GGG AAG AAG GAG GTG GAA GCG      3482
Asn Val Asp Leu Glu Ser Gln Ala Glu Gly Lys Lys Glu Val Glu Ala
        1100                1105                1110

GAT GAC GTG ATG AGG AGC GGC CCC CGG CCT ATC GTC CCA TAC AGC TCC      3530
Asp Asp Val Met Arg Ser Gly Pro Arg Pro Ile Val Pro Tyr Ser Ser
    1115                1120                1125

ATG TTC TGT TTA AGC CCC ACC AAC CTG CTC CGC CGC TTC TGC CAC TAC      3578
Met Phe Cys Leu Ser Pro Thr Asn Leu Leu Arg Arg Phe Cys His Tyr
1130                1135                1140                1145

ATC GTG ACC ATG AGG TAC TTC GAG GTG GTC ATT CTC GTG GTC ATC GCC      3626
Ile Val Thr Met Arg Tyr Phe Glu Val Val Ile Leu Val Val Ile Ala
                1150                1155                1160

TTG AGC AGC ATC GCC CTG GCT GCT GAG GAC CCA GTG CGC ACA GAC TCG      3674
Leu Ser Ser Ile Ala Leu Ala Ala Glu Asp Pro Val Arg Thr Asp Ser
            1165                1170                1175

CCC AGG AAC AAC GCT CTG AAA TAC CTG GAT TAC ATT TTC ACT GGT GTC      3722
Pro Arg Asn Asn Ala Leu Lys Tyr Leu Asp Tyr Ile Phe Thr Gly Val
        1180                1185                1190

TTT ACC TTT GAG ATG GTG ATA AAG ATG ATC GAC TTG GGA CTG CTG CTT      3770
Phe Thr Phe Glu Met Val Ile Lys Met Ile Asp Leu Gly Leu Leu Leu
    1195                1200                1205

CAC CCT GGA GCC TAT TTC CGG GAC TTG TGG AAC ATT CTG GAC TTC ATT      3818
His Pro Gly Ala Tyr Phe Arg Asp Leu Trp Asn Ile Leu Asp Phe Ile
1210                1215                1220                1225
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GTG GTC AGT GGC GCC CTG GTG GCG TTT GCT TTC TCA GGA TCC AAA GGG      3866
Val Val Ser Gly Ala Leu Val Ala Phe Ala Phe Ser Gly Ser Lys Gly
                1230                1235                1240

AAA GAC ATC AAT ACC ATC AAG TCT CTG AGA GTC CTT CGT GTC CTG CGG      3914
Lys Asp Ile Asn Thr Ile Lys Ser Leu Arg Val Leu Arg Val Leu Arg
            1245                1250                1255

CCC CTC AAG ACC ATC AAA CGG CTG CCC AAG CTC AAG GCT GTG TTT GAC      3962
Pro Leu Lys Thr Ile Lys Arg Leu Pro Lys Leu Lys Ala Val Phe Asp
        1260                1265                1270

TGT GTG GTG AAC TCC CTG AAG AAT GTC CTC AAC ATC TTG ATT GTC TAC      4010
Cys Val Val Asn Ser Leu Lys Asn Val Leu Asn Ile Leu Ile Val Tyr
    1275                1280                1285

ATG CTC TTC ATG TTC ATA TTT GCC GTC ATT GCG GTG CAG CTC TTC AAA      4058
Met Leu Phe Met Phe Ile Phe Ala Val Ile Ala Val Gln Leu Phe Lys
1290                1295                1300                1305

GGG AAG TTT TTC TAC TGC ACA GAT GAA TCC AAG GAG CTG GAG AGG GAC      4106
Gly Lys Phe Phe Tyr Cys Thr Asp Glu Ser Lys Glu Leu Glu Arg Asp
                1310                1315                1320

TGC AGG GGT CAG TAT TTG GAT TAT GAG AAG GAG GAA GTG GAA GCT CAG      4154
Cys Arg Gly Gln Tyr Leu Asp Tyr Glu Lys Glu Glu Val Glu Ala Gln
            1325                1330                1335

CCC AGG CAG TGG AAG AAA TAC GAC TTT CAC TAC GAC AAT GTG CTC TGG      4202
Pro Arg Gln Trp Lys Lys Tyr Asp Phe His Tyr Asp Asn Val Leu Trp
        1340                1345                1350

GCT CTG CTG ACG CTG TTC ACA GTG TCC ACG GGA GAA GGC TGG CCC ATG      4250
Ala Leu Leu Thr Leu Phe Thr Val Ser Thr Gly Glu Gly Trp Pro Met
    1355                1360                1365

GTG CTG AAA CAC TCC GTG GAT GCC ACC TAT GAG GAG CAG GGT CCA AGC      4298
Val Leu Lys His Ser Val Asp Ala Thr Tyr Glu Glu Gln Gly Pro Ser
1370                1375                1380                1385

CCT GGG TAC CGC ATG GAG CTG TCC ATC TTC TAC GTG GTC TAC TTT GTG      4346
Pro Gly Tyr Arg Met Glu Leu Ser Ile Phe Tyr Val Val Tyr Phe Val
                1390                1395                1400

GTC TTT CCC TTC TTC TTC GTC AAC ATC TTT GTG GCT TTG ATC ATC ATC      4394
Val Phe Pro Phe Phe Phe Val Asn Ile Phe Val Ala Leu Ile Ile Ile
            1405                1410                1415

ACC TTC CAG GAG CAG GGG GAC AAG GTG ATG TCT GAA TGC AGC CTG GAG      4442
Thr Phe Gln Glu Gln Gly Asp Lys Val Met Ser Glu Cys Ser Leu Glu
        1420                1425                1430

AAG AAC GAG AGG GCT TGC ATT GAC TTC GCC ATC AGC GCC AAA CCC CTG      4490
Lys Asn Glu Arg Ala Cys Ile Asp Phe Ala Ile Ser Ala Lys Pro Leu
    1435                1440                1445

ACA CGG TAC ATG CCC CAA AAC CGG CAG TCG TTC CAG TAT AAG ACG TGG      4538
Thr Arg Tyr Met Pro Gln Asn Arg Gln Ser Phe Gln Tyr Lys Thr Trp
1450                1455                1460                1465

ACA TTT GTG GTC TCC CCG CCC TTT GAA TAC TTC ATC ATG GCC ATG ATA      4586
Thr Phe Val Val Ser Pro Pro Phe Glu Tyr Phe Ile Met Ala Met Ile
                1470                1475                1480

GCC CTC AAC ACT GTG GTG CTG ATG ATG AAG TTC TAT GAT GCA CCC TAT      4634
Ala Leu Asn Thr Val Val Leu Met Met Lys Phe Tyr Asp Ala Pro Tyr
            1485                1490                1495
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GAG TAC GAG CTG ATG CTG AAA TGC CTG AAC ATC GTG TTC ACA TCC ATG      4682
Glu Tyr Glu Leu Met Leu Lys Cys Leu Asn Ile Val Phe Thr Ser Met
        1500                1505                1510

TTC TCC ATG GAA TGC GTG CTG AAG ATC ATC GCC TTT GGG GTG CTG AAC      4730
Phe Ser Met Glu Cys Val Leu Lys Ile Ile Ala Phe Gly Val Leu Asn
    1515                1520                1525

TAT TTC AGA GAT GCC TGG AAT GTC TTT GAC TTT GTC ACT GTG TTG GGA      4778
Tyr Phe Arg Asp Ala Trp Asn Val Phe Asp Phe Val Thr Val Leu Gly
1530                1535                1540                1545

AGT ATT ACT GAT ATT TTA GTA ACA GAG ATT GCG GAA ACG AAC AAT TTC      4826
Ser Ile Thr Asp Ile Leu Val Thr Glu Ile Ala Glu Thr Asn Asn Phe
                1550                1555                1560

ATC AAC CTC AGC TTC CTC CGC CTC TTT CGA GCT GCG CGG CTG ATC AAG      4874
Ile Asn Leu Ser Phe Leu Arg Leu Phe Arg Ala Ala Arg Leu Ile Lys
            1565                1570                1575

CTG CTC CGC CAG GGC TAC ACC ATC CGC ATC CTG CTG TGG ACC TTT GTC      4922
Leu Leu Arg Gln Gly Tyr Thr Ile Arg Ile Leu Leu Trp Thr Phe Val
        1580                1585                1590

CAG TCC TTC AAG GCC CTG CCC TAC GTG TGT CTG CTC ATT GCC ATG CTG      4970
Gln Ser Phe Lys Ala Leu Pro Tyr Val Cys Leu Leu Ile Ala Met Leu
    1595                1600                1605

TTC TTC ATC TAC GCC ATC ATC GGC ATG CAG GTG TTT GGG AAT ATT GCC      5018
Phe Phe Ile Tyr Ala Ile Ile Gly Met Gln Val Phe Gly Asn Ile Ala
1610                1615                1620                1625

CTG GAT GAT GAC ACC AGC ATC AAC CGC CAC AAC AAC TTC CGG ACG TTT      5066
Leu Asp Asp Asp Thr Ser Ile Asn Arg His Asn Asn Phe Arg Thr Phe
                1630                1635                1640

TTG CAA GCC CTG ATG CTG CTG TTC AGG AGC GCC ACG GGG GAG GCC TGG      5114
Leu Gln Ala Leu Met Leu Leu Phe Arg Ser Ala Thr Gly Glu Ala Trp
            1645                1650                1655

CAC GAG ATC ATG CTG TCC TGC CTG AGC AAC CAG GCC TGT GAT GAG CAG      5162
His Glu Ile Met Leu Ser Cys Leu Ser Asn Gln Ala Cys Asp Glu Gln
        1660                1665                1670

GCC AAT GCC ACC GAG TGT GGA AGT GAC TTT GCC TAC TTC TAC TTC GTC      5210
Ala Asn Ala Thr Glu Cys Gly Ser Asp Phe Ala Tyr Phe Tyr Phe Val
    1675                1680                1685

TCC TTC ATC TTC CTG TGC TCC TTT CTG ATG TTG AAC CTC TTT GTG GCT      5258
Ser Phe Ile Phe Leu Cys Ser Phe Leu Met Leu Asn Leu Phe Val Ala
1690                1695                1700                1705

GTG ATC ATG GAC AAT TTT GAG TAC CTC ACG CGG GAC TCT TCC ATC CTA      5306
Val Ile Met Asp Asn Phe Glu Tyr Leu Thr Arg Asp Ser Ser Ile Leu
                1710                1715                1720

GGT CCT CAC CAC TTG GAT GAG TTC ATC CGG GTC TGG GCT GAA TAC GAC      5354
Gly Pro His His Leu Asp Glu Phe Ile Arg Val Trp Ala Glu Tyr Asp
            1725                1730                1735

CCG GCT GCG TGT GGG CGC ATC AGT TAC AAT GAC ATG TTT GAG ATG CTG      5402
Pro Ala Ala Cys Gly Arg Ile Ser Tyr Asn Asp Met Phe Glu Met Leu
        1740                1745                1750

AAA CAC ATG TCC CCG CCT CTG GGG CTG GGG AAG AAA TGC CCT GCT CGA      5450
Lys His Met Ser Pro Pro Leu Gly Leu Gly Lys Lys Cys Pro Ala Arg
    1755                1760                1765
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GTT GCT TAC AAG CGC CTG GTT CGC ATG AAC ATG CCC ATC TCC AAC GAG      5498
Val Ala Tyr Lys Arg Leu Val Arg Met Asn Met Pro Ile Ser Asn Glu
1770                1775                1780                1785

GAC ATG ACT GTT CAC TTC ACG TCC ACG CTG ATG GCC CTC ATC CGG ACG      5546
Asp Met Thr Val His Phe Thr Ser Thr Leu Met Ala Leu Ile Arg Thr
                1790                1795                1800

GCA CTG GAG ATC AAG CTG GCC CCA GCT GGG ACA AAG CAG CAT CAG TGT      5594
Ala Leu Glu Ile Lys Leu Ala Pro Ala Gly Thr Lys Gln His Gln Cys
            1805                1810                1815

GAC GCG GAG TTG AGG AAG GAG ATT TCC GTT GTG TGG GCC AAT CTG CCC      5642
Asp Ala Glu Leu Arg Lys Glu Ile Ser Val Val Trp Ala Asn Leu Pro
        1820                1825                1830

CAG AAG ACT TTG GAC TTG CTG GTA CCA CCC CAT AAG CCT GAT GAG ATG      5690
Gln Lys Thr Leu Asp Leu Leu Val Pro Pro His Lys Pro Asp Glu Met
    1835                1840                1845

ACA GTG GGG AAG GTT TAT GCA GCT CTG ATG ATA TTT GAC TTC TAC AAG      5738
Thr Val Gly Lys Val Tyr Ala Ala Leu Met Ile Phe Asp Phe Tyr Lys
1850                1855                1860                1865

CAG AAC AAA ACC ACC AGA GAC CAG ATG CAG CAG GCT CCT GGA GGC CTC      5786
Gln Asn Lys Thr Thr Arg Asp Gln Met Gln Gln Ala Pro Gly Gly Leu
                1870                1875                1880

TCC CAG ATG GGT CCT GTG TCC CTG TTC CAC CCT CTG AAG GCC ACC CTG      5834
Ser Gln Met Gly Pro Val Ser Leu Phe His Pro Leu Lys Ala Thr Leu
            1885                1890                1895

GAG CAG ACA CAG CCG GCT GTG CTC CGA GGA GCC CGG GTT TTC CTT CGA      5882
Glu Gln Thr Gln Pro Ala Val Leu Arg Gly Ala Arg Val Phe Leu Arg
        1900                1905                1910

CAG AAG AGT TCC ACC TCC CTC AGC AAT GGC GGG GCC ATA CAA AAC CAA      5930
Gln Lys Ser Ser Thr Ser Leu Ser Asn Gly Gly Ala Ile Gln Asn Gln
    1915                1920                1925

GAG AGT GGC ATC AAA GAG TCT GTC TCC TGG GGC ACT CAA AGG ACC CAG      5978
Glu Ser Gly Ile Lys Glu Ser Val Ser Trp Gly Thr Gln Arg Thr Gln
1930                1935                1940                1945

GAT GCA CCC CAT GAG GCC AGG CCA CCC CTG GAG CGT GGC CAC TCC ACA      6026
Asp Ala Pro His Glu Ala Arg Pro Pro Leu Glu Arg Gly His Ser Thr
                1950                1955                1960

GAG ATC CCT GTG GGG CGG TCA GGA GCA CTG GCT GTG GAC GTT CAG ATG      6074
Glu Ile Pro Val Gly Arg Ser Gly Ala Leu Ala Val Asp Val Gln Met
            1965                1970                1975

CAG AGC ATA ACC CGG AGG GGC CCT GAT GGG GAG CCC CAG CCT GGG CTG      6122
Gln Ser Ile Thr Arg Arg Gly Pro Asp Gly Glu Pro Gln Pro Gly Leu
        1980                1985                1990

GAG AGC CAG GGT CGA GCG GCC TCC ATG CCC CGC CTT GCG GCC GAG ACT      6170
Glu Ser Gln Gly Arg Ala Ala Ser Met Pro Arg Leu Ala Ala Glu Thr
    1995                2000                2005

CAG CCC GTC ACA GAT GCC AGC CCC ATG AAG CGC TCC ATC TCC ACG CTG      6218
Gln Pro Val Thr Asp Ala Ser Pro Met Lys Arg Ser Ile Ser Thr Leu
2010                2015                2020                2025

GCC CAG CGG CCC CGT GGG ACT CAT CTT TGC AGC ACC ACC CCG GAC CGC      6266
Ala Gln Arg Pro Arg Gly Thr His Leu Cys Ser Thr Thr Pro Asp Arg
                2030                2035                2040
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(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1437

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 1435..1546

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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ATG GTC CAG AAG ACC AGC ATG TCC CGG GGC CCT TAC CCA CCC TCC CAG      48
Met Val Gln Lys Thr Ser Met Ser Arg Gly Pro Tyr Pro Pro Ser Gln
 1               5                  10                  15

GAG ATC CCC ATG GAG GTC TTC GAC CCC AGC CCG CAG GGC AAA TAC AGC      96
Glu Ile Pro Met Glu Val Phe Asp Pro Ser Pro Gln Gly Lys Tyr Ser
            20                  25                  30

AAG AGG AAA GGG CGA TTC AAA CGG TCA GAT GGG AGC ACG TCC TCG GAT     144
Lys Arg Lys Gly Arg Phe Lys Arg Ser Asp Gly Ser Thr Ser Ser Asp
        35                  40                  45

ACC ACA TCC AAC AGC TTT GTC CGC CAG GGC TCA GCG GAG TCC TAC ACC     192
Thr Thr Ser Asn Ser Phe Val Arg Gln Gly Ser Ala Glu Ser Tyr Thr
    50                  55                  60

AGC CGT CCA TCA GAC TCT GAT GTA TCT CTG GAG GAG GAC CGG GAA GCC     240
Ser Arg Pro Ser Asp Ser Asp Val Ser Leu Glu Glu Asp Arg Glu Ala
65                  70                  75                  80

TTA AGG AAG GAA GCA GAG CGC CAG GCA TTA GCG CAG CTC GAG AAG GCC     288
Leu Arg Lys Glu Ala Glu Arg Gln Ala Leu Ala Gln Leu Glu Lys Ala
                85                  90                  95

AAG ACC AAG CCA GTG GCA TTT GCT GTG CGG ACA AAT GTT GGC TAC AAT     336
Lys Thr Lys Pro Val Ala Phe Ala Val Arg Thr Asn Val Gly Tyr Asn
            100                 105                 110

CCG TCT CCA GGG GAT GAG GTG CCT GTG CAG GGA GTG GCC ATC ACC TTC     384
Pro Ser Pro Gly Asp Glu Val Pro Val Gln Gly Val Ala Ile Thr Phe
        115                 120                 125

GAG CCC AAA GAC TTC CTG CAC ATC AAG GAG AAA TAC AAT AAT GAC TGG     432
Glu Pro Lys Asp Phe Leu His Ile Lys Glu Lys Tyr Asn Asn Asp Trp
    130                 135                 140

TGG ATC GGG CGG CTG GTG AAG GAG GGC TGT GAG GTT GGC TTC ATT CCC     480
Trp Ile Gly Arg Leu Val Lys Glu Gly Cys Glu Val Gly Phe Ile Pro
145                 150                 155                 160

AGC CCC GTC AAA CTG GAC AGC CTT CGC CTG CTG CAG GAA CAG AAG CTG     528
Ser Pro Val Lys Leu Asp Ser Leu Arg Leu Leu Gln Glu Gln Lys Leu
                165                 170                 175

CGC CAG AAC CGC CTC GGC TCC AGC AAA TCA GGC GAT AAC TCC AGT TCC     576
Arg Gln Asn Arg Leu Gly Ser Ser Lys Ser Gly Asp Asn Ser Ser Ser
            180                 185                 190

AGT CTG GGA GAT GTG GTG ACT GGC ACC CGC CGC CCC ACA CCC CCT GCC     624
Ser Leu Gly Asp Val Val Thr Gly Thr Arg Arg Pro Thr Pro Pro Ala
        195                 200                 205
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AGT GCC AAA CAG AAG CAG AAG TCG ACA GAG CAT GTG CCC CCC TAT GAC        672
Ser Ala Lys Gln Lys Gln Lys Ser Thr Glu His Val Pro Pro Tyr Asp
    210                 215                 220

GTG GTG CCT TCC ATG AGG CCC ATC ATC CTG GTG GGA CCG TCG CTC AAG        720
Val Val Pro Ser Met Arg Pro Ile Ile Leu Val Gly Pro Ser Leu Lys
225                 230                 235                 240

GGC TAC GAG GTT ACA GAC ATG ATG CAG AAA GCT TTA TTT GAC TTC TTG        768
Gly Tyr Glu Val Thr Asp Met Met Gln Lys Ala Leu Phe Asp Phe Leu
                245                 250                 255

AAG CAT CGG TTT GAT GGC AGG ATC TCC ATC ACT CGT GTG ACG GCA GAT        816
Lys His Arg Phe Asp Gly Arg Ile Ser Ile Thr Arg Val Thr Ala Asp
            260                 265                 270

ATT TCC CTG GCT AAG CGC TCA GTT CTC AAC AAC CCC AGC AAA CAC ATC        864
Ile Ser Leu Ala Lys Arg Ser Val Leu Asn Asn Pro Ser Lys His Ile
        275                 280                 285

ATC ATT GAG CGC TCC AAC ACA CGC TCC AGC CTG GCT GAG GTG CAG AGT        912
Ile Ile Glu Arg Ser Asn Thr Arg Ser Ser Leu Ala Glu Val Gln Ser
    290                 295                 300

GAA ATC GAG CGA ATC TTC GAG CTG GCC CGG ACC CTT CAG TTG GTC GCT        960
Glu Ile Glu Arg Ile Phe Glu Leu Ala Arg Thr Leu Gln Leu Val Ala
305                 310                 315                 320

CTG GAT GCT GAC ACC ATC AAT CAC CCA GCC CAG CTG TCC AAG ACC TCG       1008
Leu Asp Ala Asp Thr Ile Asn His Pro Ala Gln Leu Ser Lys Thr Ser
                325                 330                 335

CTG GCC CCC ATC ATT GTT TAC ATC AAG ATC ACC TCT CCC AAG GTA CTT       1056
Leu Ala Pro Ile Ile Val Tyr Ile Lys Ile Thr Ser Pro Lys Val Leu
            340                 345                 350

CAA AGG CTC ATC AAG TCC CGA GGA AAG TCT CAG TCC AAA CAC CTC AAT       1104
Gln Arg Leu Ile Lys Ser Arg Gly Lys Ser Gln Ser Lys His Leu Asn
        355                 360                 365

GTC CAA ATA GCG GCC TCG GAA AAG CTG GCA CAG TGC CCC CCT GAA ATG       1152
Val Gln Ile Ala Ala Ser Glu Lys Leu Ala Gln Cys Pro Pro Glu Met
    370                 375                 380

TTT GAC ATC ATC CTG GAT GAG AAC CAA TTG GAG GAT GCC TGC GAG CAT       1200
Phe Asp Ile Ile Leu Asp Glu Asn Gln Leu Glu Asp Ala Cys Glu His
385                 390                 395                 400

CTG GCG GAG TAC TTG GAA GCC TAT TGG AAG GCC ACA CAC CCG CCC AGC       1248
Leu Ala Glu Tyr Leu Glu Ala Tyr Trp Lys Ala Thr His Pro Pro Ser
                405                 410                 415

AGC ACG CCA CCC AAT CCG CTG CTG AAC CGC ACC ATG GCT ACC GCA GCC       1296
Ser Thr Pro Pro Asn Pro Leu Leu Asn Arg Thr Met Ala Thr Ala Ala
            420                 425                 430

CTG GCT GCC AGC CCT GCC CCT GTC TCC AAC CTC CAG GTA CAG GTG CTC       1344
Leu Ala Ala Ser Pro Ala Pro Val Ser Asn Leu Gln Val Gln Val Leu
        435                 440                 445

ACC TCG CTC AGG AGA AAC CTC GGC TTC TGG GGC GGG CTG GAG TCC TCA       1392
Thr Ser Leu Arg Arg Asn Leu Gly Phe Trp Gly Gly Leu Glu Ser Ser
    450                 455                 460

CAG CGG GGC AGT GTG GTG CCC CAG GAG CAG GAA CAT GCC ATG TAGTGGGCGC    1444
Gln Arg Gly Ser Val Val Pro Gln Glu Gln Glu His Ala Met
465                 470                 475
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CCTGCCCGTC TTCCCTCCTG CTCTGGGGTC GGAACTGGAG TGCAGGGAAC ATGGAGGAGG 1504

AAGGGAAGAG CTTTATTTTG TAAAAAAATA AGATGAGCGG CA 1546

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1851 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1797
(D) OTHER INFORMATION: /standard_name= "Beta-3"

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 1795..1851

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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ATG GTC CAG AAG ACC AGC ATG TCC CGG GGC CCT TAC CCA CCC TCC CAG      48
Met Val Gln Lys Thr Ser Met Ser Arg Gly Pro Tyr Pro Pro Ser Gln
  1               5                  10                  15

GAG ATC CCC ATG GGA GTC TTC GAC CCC AGC CCG CAG GGC AAA TAC AGC      96
Glu Ile Pro Met Gly Val Phe Asp Pro Ser Pro Gln Gly Lys Tyr Ser
            20                  25                  30

AAG AGG AAA GGG CGA TTC AAA CGG TCA GAT GGG AGC ACG TCC TCG GAT     144
Lys Arg Lys Gly Arg Phe Lys Arg Ser Asp Gly Ser Thr Ser Ser Asp
        35                  40                  45

ACC ACA TCC AAC AGC TTT GTC CGC CAG GGC TCA GCG GAG TCC TAC ACC     192
Thr Thr Ser Asn Ser Phe Val Arg Gln Gly Ser Ala Glu Ser Tyr Thr
    50                  55                  60

AGC CGT CCA TCA GAC TCT GAT GTA TCT CTG GAG GAG GAC CGG GAA GCC     240
Ser Arg Pro Ser Asp Ser Asp Val Ser Leu Glu Glu Asp Arg Glu Ala
65                  70                  75                  80

TTA AGG AAG GAA GCA GAG CGC CAG GCA TTA GCG CAG CTC GAG AAG GCC     288
Leu Arg Lys Glu Ala Glu Arg Gln Ala Leu Ala Gln Leu Glu Lys Ala
                85                  90                  95

AAG ACC AAG CCA GTG GCA TTT GCT GTG CGG ACA AAT GTT GGC TAC AAT     336
Lys Thr Lys Pro Val Ala Phe Ala Val Arg Thr Asn Val Gly Tyr Asn
            100                 105                 110

CCG TCT CCA GGG GAT GAG GTG CCT GTG CAG GGA GTG GCC ATC ACC TTC     384
Pro Ser Pro Gly Asp Glu Val Pro Val Gln Gly Val Ala Ile Thr Phe
        115                 120                 125

GAG CCC AAA GAC TTC CTG CAC ATC AAG GAG AAA TAC AAT AAT GAC TGG     432
Glu Pro Lys Asp Phe Leu His Ile Lys Glu Lys Tyr Asn Asn Asp Trp
    130                 135                 140

TGG ATC GGG CGG CTG GTG AAG GAG GGC TGT GAG GTT GGC TTC ATT CCC     480
Trp Ile Gly Arg Leu Val Lys Glu Gly Cys Glu Val Gly Phe Ile Pro
145                 150                 155                 160
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AGC CCC GTC AAA CTG GAC AGC CTT CGC CTG CTG CAG GAA CAG AAG CTG          528
Ser Pro Val Lys Leu Asp Ser Leu Arg Leu Leu Gln Glu Gln Lys Leu
                165                 170                 175

CGC CAG AAC CGC CTC GGC TCC AGC AAA TCA GGC GAT AAC TCC AGT TCC          576
Arg Gln Asn Arg Leu Gly Ser Ser Lys Ser Gly Asp Asn Ser Ser Ser
            180                 185                 190

AGT CTG GGA GAT GTG GTG ACT GGC ACC CGC CGC CCC ACA CCC CCT GCC          624
Ser Leu Gly Asp Val Val Thr Gly Thr Arg Arg Pro Thr Pro Pro Ala
        195                 200                 205

AGT GCC AAA CAG AAG CAG AAG TCG ACA GAG CAT GTG CCC CCC TAT GAC          672
Ser Ala Lys Gln Lys Gln Lys Ser Thr Glu His Val Pro Pro Tyr Asp
    210                 215                 220

GTG GTG CCT TCC ATG AGG CCC ATC ATC CTG GTG GGA CCG TCG CTC AAG          720
Val Val Pro Ser Met Arg Pro Ile Ile Leu Val Gly Pro Ser Leu Lys
225                 230                 235                 240

GGC TAC GAG GTT ACA GAC ATG ATG CAG AAA GCT TTA TTT GAC TTC TTG          768
Gly Tyr Glu Val Thr Asp Met Met Gln Lys Ala Leu Phe Asp Phe Leu
                245                 250                 255

AAG CAT CGG TTT GAT GGC AGG ATC TCC ATC ACT CGT GTG ACG GCA GAT          816
Lys His Arg Phe Asp Gly Arg Ile Ser Ile Thr Arg Val Thr Ala Asp
            260                 265                 270

ATT TCC CTG GCT AAG CGC TCA GTT CTC AAC AAC CCC AGC AAA CAC ATC          864
Ile Ser Leu Ala Lys Arg Ser Val Leu Asn Asn Pro Ser Lys His Ile
        275                 280                 285

ATC ATT GAG CGC TCC AAC ACA CGC TCC AGC CTG GCT GAG GTG CAG AGT          912
Ile Ile Glu Arg Ser Asn Thr Arg Ser Ser Leu Ala Glu Val Gln Ser
    290                 295                 300

GAA ATC GAG CGA ATC TTC GAG CTG GCC CGG ACC CTT CAG TTG GTC GCT          960
Glu Ile Glu Arg Ile Phe Glu Leu Ala Arg Thr Leu Gln Leu Val Ala
305                 310                 315                 320

CTG GAT GCT GAC ACC ATC AAT CAC CCA GCC CAG CTG TCC AAG ACC TCG         1008
Leu Asp Ala Asp Thr Ile Asn His Pro Ala Gln Leu Ser Lys Thr Ser
                325                 330                 335

CTG GCC CCC ATC ATT GTT TAC ATC AAG ATC ACC TCT CCC AAG GTA CTT         1056
Leu Ala Pro Ile Ile Val Tyr Ile Lys Ile Thr Ser Pro Lys Val Leu
            340                 345                 350

CAA AGG CTC ATC AAG TCC CGA GGA AAG TCT CAG TCC AAA CAC CTC AAT         1104
Gln Arg Leu Ile Lys Ser Arg Gly Lys Ser Gln Ser Lys His Leu Asn
        355                 360                 365

GTC CAA ATA GCG GCC TCG GAA AAG CTG GCA CAG TGC CCC CCT GAA ATG         1152
Val Gln Ile Ala Ala Ser Glu Lys Leu Ala Gln Cys Pro Pro Glu Met
    370                 375                 380

TTT GAC ATC ATC CTG GAT GAG AAC CAA TTG GAG GAT GCC TGC GAG CAT         1200
Phe Asp Ile Ile Leu Asp Glu Asn Gln Leu Glu Asp Ala Cys Glu His
385                 390                 395                 400

CTG GCG GAG TAC TTG GAA GCC TAT TGG AAG GCC ACA CAC CCG CCC AGC         1248
Leu Ala Glu Tyr Leu Glu Ala Tyr Trp Lys Ala Thr His Pro Pro Ser
                405                 410                 415

AGC ACG CCA CCC AAT CCG CTG CTG AAC CGC ACC ATG GCT ACC GCA GCC         1296
Ser Thr Pro Pro Asn Pro Leu Leu Asn Arg Thr Met Ala Thr Ala Ala
            420                 425                 430
```

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CTG GCT GCC AGC CCT GCC CCT GTC TCC AAC CTC CAG GGA CCC TAC CTT      1344
Leu Ala Ala Ser Pro Ala Pro Val Ser Asn Leu Gln Gly Pro Tyr Leu
        435                 440                 445

GCT TCC GGG GAC CAG CCA CTG GAA CGG GCC ACC GGG GAG CAC GCC AGC      1392
Ala Ser Gly Asp Gln Pro Leu Glu Arg Ala Thr Gly Glu His Ala Ser
    450                 455                 460

ATG CAC GAG TAC CCA GGG GAG CTG GGC CAG CCC CCA GGC CTT TAC CCC      1440
Met His Glu Tyr Pro Gly Glu Leu Gly Gln Pro Pro Gly Leu Tyr Pro
465                 470                 475                 480

AGC AGC CAC CCA CCA GGC CGG GCA GGC ACG CTA CGG GCA CTG TCC CGC      1488
Ser Ser His Pro Pro Gly Arg Ala Gly Thr Leu Arg Ala Leu Ser Arg
                485                 490                 495

CAA GAC ACT TTT GAT GCC GAC ACC CCC GGC AGC CGA AAC TCT GCC TAC      1536
Gln Asp Thr Phe Asp Ala Asp Thr Pro Gly Ser Arg Asn Ser Ala Tyr
            500                 505                 510

ACG GAG CTG GGA GAC TCA TGT GTG GAC ATG GAG ACT GAC CCC TCA GAG      1584
Thr Glu Leu Gly Asp Ser Cys Val Asp Met Glu Thr Asp Pro Ser Glu
        515                 520                 525

GGG CCA GGG CTT GGA GAC CCT GCA GGG GGC GGC ACG CCC CCA GCC CGA      1632
Gly Pro Gly Leu Gly Asp Pro Ala Gly Gly Gly Thr Pro Pro Ala Arg
    530                 535                 540

CAG GGA TCC TGG GAG GAC GAG GAA GAA GAC TAT GAG GAA GAG CTG ACC      1680
Gln Gly Ser Trp Glu Asp Glu Glu Glu Asp Tyr Glu Glu Glu Leu Thr
545                 550                 555                 560

GAC AAC CGG AAC CGG GGC CGG AAT AAG GCC CGC TAC TGC GCT GAG GGT      1728
Asp Asn Arg Asn Arg Gly Arg Asn Lys Ala Arg Tyr Cys Ala Glu Gly
                565                 570                 575

GGG GGT CCA GTT TTG GGG CGC AAC AAG AAT GAG CTG GAG GGC TGG GGA      1776
Gly Gly Pro Val Leu Gly Arg Asn Lys Asn Glu Leu Glu Gly Trp Gly
            580                 585                 590

CGA GGC GTC TAC ATT CGC TGAGAGGCAG GGGCCACACG GCGGGAGGAA             1824
Arg Gly Val Tyr Ile Arg
        595

GGGCTCTGAG CCCAGGGGAG GGGAGGG                                       1851
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3600 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 35..3310
(D) OTHER INFORMATION: /standard_name= "Alpha-2"

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..34

(ix) FEATURE:
(A) NAME/KEY: 3'UTR

(B) LOCATION: 3308..3600

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
GCGGGGGAGG GGGCATTGAT CTTCGATCGC GAAG ATG GCT GCT GGC TGC CTG          52
                                        Met Ala Ala Gly Cys Leu
                                          1               5

CTG GCC TTG ACT CTG ACA CTT TTC CAA TCT TTG CTC ATC GGC CCC TCG        100
Leu Ala Leu Thr Leu Thr Leu Phe Gln Ser Leu Leu Ile Gly Pro Ser
            10                  15                  20

TCG GAG GAG CCG TTC CCT TCG GCC GTC ACT ATC AAA TCA TGG GTG GAT        148
Ser Glu Glu Pro Phe Pro Ser Ala Val Thr Ile Lys Ser Trp Val Asp
        25                  30                  35

AAG ATG CAA GAA GAC CTT GTC ACA CTG GCA AAA ACA GCA AGT GGA GTC        196
Lys Met Gln Glu Asp Leu Val Thr Leu Ala Lys Thr Ala Ser Gly Val
    40                  45                  50

AAT CAG CTT GTT GAT ATT TAT GAG AAA TAT CAA GAT TTG TAT ACT GTG        244
Asn Gln Leu Val Asp Ile Tyr Glu Lys Tyr Gln Asp Leu Tyr Thr Val
55                  60                  65                  70

GAA CCA AAT AAT GCA CGC CAG CTG GTA GAA ATT GCA GCC AGG GAT ATT        292
Glu Pro Asn Asn Ala Arg Gln Leu Val Glu Ile Ala Ala Arg Asp Ile
                75                  80                  85

GAG AAA CTT CTG AGC AAC AGA TCT AAA GCC CTG GTG AGC CTG GCA TTG        340
Glu Lys Leu Leu Ser Asn Arg Ser Lys Ala Leu Val Ser Leu Ala Leu
            90                  95                  100

GAA GCG GAG AAA GTT CAA GCA GCT CAC CAG TGG AGA GAA GAT TTT GCA        388
Glu Ala Glu Lys Val Gln Ala Ala His Gln Trp Arg Glu Asp Phe Ala
        105                 110                 115

AGC AAT GAA GTT GTC TAC TAC AAT GCA AAG GAT GAT CTC GAT CCT GAG        436
Ser Asn Glu Val Val Tyr Tyr Asn Ala Lys Asp Asp Leu Asp Pro Glu
    120                 125                 130

AAA AAT GAC AGT GAG CCA GGC AGC CAG AGG ATA AAA CCT GTT TTC ATT        484
Lys Asn Asp Ser Glu Pro Gly Ser Gln Arg Ile Lys Pro Val Phe Ile
135                 140                 145                 150

GAA GAT GCT AAT TTT GGA CGA CAA ATA TCT TAT CAG CAC GCA GCA GTC        532
Glu Asp Ala Asn Phe Gly Arg Gln Ile Ser Tyr Gln His Ala Ala Val
                155                 160                 165

CAT ATT CCT ACT GAC ATC TAT GAG GGC TCA ACA ATT GTG TTA AAT GAA        580
His Ile Pro Thr Asp Ile Tyr Glu Gly Ser Thr Ile Val Leu Asn Glu
            170                 175                 180

CTC AAC TGG ACA AGT GCC TTA GAT GAA GTT TTC AAA AAG AAT CGC GAG        628
Leu Asn Trp Thr Ser Ala Leu Asp Glu Val Phe Lys Lys Asn Arg Glu
        185                 190                 195

GAA GAC CCT TCA TTA TTG TGG CAG GTT TTT GGC AGT GCC ACT GGC CTA        676
Glu Asp Pro Ser Leu Leu Trp Gln Val Phe Gly Ser Ala Thr Gly Leu
    200                 205                 210

GCT CGA TAT TAT CCA GCT TCA CCA TGG GTT GAT AAT AGT AGA ACT CCA        724
Ala Arg Tyr Tyr Pro Ala Ser Pro Trp Val Asp Asn Ser Arg Thr Pro
215                 220                 225                 230

AAT AAG ATT GAC CTT TAT GAT GTA CGC AGA AGA CCA TGG TAC ATC CAA        772
Asn Lys Ile Asp Leu Tyr Asp Val Arg Arg Arg Pro Trp Tyr Ile Gln
                235                 240                 245
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GGA GCT GCA TCT CCT AAA GAC ATG CTT ATT CTG GTG GAT GTG AGT GGA      820
Gly Ala Ala Ser Pro Lys Asp Met Leu Ile Leu Val Asp Val Ser Gly
            250                 255                 260

AGT GTT AGT GGA TTG ACA CTT AAA CTG ATC CGA ACA TCT GTC TCC GAA      868
Ser Val Ser Gly Leu Thr Leu Lys Leu Ile Arg Thr Ser Val Ser Glu
        265                 270                 275

ATG TTA GAA ACC CTC TCA GAT GAT GAT TTC GTG AAT GTA GCT TCA TTT      916
Met Leu Glu Thr Leu Ser Asp Asp Asp Phe Val Asn Val Ala Ser Phe
    280                 285                 290

AAC AGC AAT GCT CAG GAT GTA AGC TGT TTT CAG CAC CTT GTC CAA GCA      964
Asn Ser Asn Ala Gln Asp Val Ser Cys Phe Gln His Leu Val Gln Ala
295                 300                 305                 310

AAT GTA AGA AAT AAA AAA GTG TTG AAA GAC GCG GTG AAT AAT ATC ACA     1012
Asn Val Arg Asn Lys Lys Val Leu Lys Asp Ala Val Asn Asn Ile Thr
                315                 320                 325

GCC AAA GGA ATT ACA GAT TAT AAG AAG GGC TTT AGT TTT GCT TTT GAA     1060
Ala Lys Gly Ile Thr Asp Tyr Lys Lys Gly Phe Ser Phe Ala Phe Glu
            330                 335                 340

CAG CTG CTT AAT TAT AAT GTT TCC AGA GCA AAC TGC AAT AAG ATT ATT     1108
Gln Leu Leu Asn Tyr Asn Val Ser Arg Ala Asn Cys Asn Lys Ile Ile
        345                 350                 355

ATG CTA TTC ACG GAT GGA GGA GAA GAG AGA GCC CAG GAG ATA TTT AAC     1156
Met Leu Phe Thr Asp Gly Gly Glu Glu Arg Ala Gln Glu Ile Phe Asn
    360                 365                 370

AAA TAC AAT AAA GAT AAA AAA GTA CGT GTA TTC AGG TTT TCA GTT GGT     1204
Lys Tyr Asn Lys Asp Lys Lys Val Arg Val Phe Arg Phe Ser Val Gly
375                 380                 385                 390

CAA CAC AAT TAT GAG AGA GGA CCT ATT CAG TGG ATG GCC TGT GAA AAC     1252
Gln His Asn Tyr Glu Arg Gly Pro Ile Gln Trp Met Ala Cys Glu Asn
                395                 400                 405

AAA GGT TAT TAT TAT GAA ATT CCT TCC ATT GGT GCA ATA AGA ATC AAT     1300
Lys Gly Tyr Tyr Tyr Glu Ile Pro Ser Ile Gly Ala Ile Arg Ile Asn
            410                 415                 420

ACT CAG GAA TAT TTG GAT GTT TTG GGA AGA CCA ATG GTT TTA GCA GGA     1348
Thr Gln Glu Tyr Leu Asp Val Leu Gly Arg Pro Met Val Leu Ala Gly
        425                 430                 435

GAC AAA GCT AAG CAA GTC CAA TGG ACA AAT GTG TAC CTG GAT GCA TTG     1396
Asp Lys Ala Lys Gln Val Gln Trp Thr Asn Val Tyr Leu Asp Ala Leu
    440                 445                 450

GAA CTG GGA CTT GTC ATT ACT GGA ACT CTT CCG GTC TTC AAC ATA ACC     1444
Glu Leu Gly Leu Val Ile Thr Gly Thr Leu Pro Val Phe Asn Ile Thr
455                 460                 465                 470

GGC CAA TTT GAA AAT AAG ACA AAC TTA AAG AAC CAG CTG ATT CTT GGT     1492
Gly Gln Phe Glu Asn Lys Thr Asn Leu Lys Asn Gln Leu Ile Leu Gly
                475                 480                 485

GTG ATG GGA GTA GAT GTG TCT TTG GAA GAT ATT AAA AGA CTG ACA CCA     1540
Val Met Gly Val Asp Val Ser Leu Glu Asp Ile Lys Arg Leu Thr Pro
            490                 495                 500

CGT TTT ACA CTG TGC CCC AAT GGG TAT TAC TTT GCA ATC GAT CCT AAT     1588
Arg Phe Thr Leu Cys Pro Asn Gly Tyr Tyr Phe Ala Ile Asp Pro Asn
        505                 510                 515
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GGT TAT GTT TTA TTA CAT CCA AAT CTT CAG CCA AAG AAC CCC AAA TCT      1636
Gly Tyr Val Leu Leu His Pro Asn Leu Gln Pro Lys Asn Pro Lys Ser
    520                 525                 530

CAG GAG CCA GTA ACA TTG GAT TTC CTT GAT GCA GAG TTA GAG AAT GAT      1684
Gln Glu Pro Val Thr Leu Asp Phe Leu Asp Ala Glu Leu Glu Asn Asp
535                 540                 545                 550

ATT AAA GTG GAG ATT CGA AAT AAG ATG ATT GAT GGG GAA AGT GGA GAA      1732
Ile Lys Val Glu Ile Arg Asn Lys Met Ile Asp Gly Glu Ser Gly Glu
                555                 560                 565

AAA ACA TTC AGA ACT CTG GTT AAA TCT CAA GAT GAG AGA TAT ATT GAC      1780
Lys Thr Phe Arg Thr Leu Val Lys Ser Gln Asp Glu Arg Tyr Ile Asp
            570                 575                 580

AAA GGA AAC AGG ACA TAC ACA TGG ACA CCT GTC AAT GGC ACA GAT TAC      1828
Lys Gly Asn Arg Thr Tyr Thr Trp Thr Pro Val Asn Gly Thr Asp Tyr
        585                 590                 595

AGT TTG GCC TTG GTA TTA CCA ACC TAC AGT TTT TAC TAT ATA AAA GCC      1876
Ser Leu Ala Leu Val Leu Pro Thr Tyr Ser Phe Tyr Tyr Ile Lys Ala
    600                 605                 610

AAA CTA GAA GAG ACA ATA ACT CAG GCC AGA TCA AAA AAG GGC AAA ATG      1924
Lys Leu Glu Glu Thr Ile Thr Gln Ala Arg Ser Lys Lys Gly Lys Met
615                 620                 625                 630

AAG GAT TCG GAA ACC CTG AAG CCA GAT AAT TTT GAA GAA TCT GGC TAT      1972
Lys Asp Ser Glu Thr Leu Lys Pro Asp Asn Phe Glu Glu Ser Gly Tyr
                635                 640                 645

ACA TTC ATA GCA CCA AGA GAT TAC TGC AAT GAC CTG AAA ATA TCG GAT      2020
Thr Phe Ile Ala Pro Arg Asp Tyr Cys Asn Asp Leu Lys Ile Ser Asp
            650                 655                 660

AAT AAC ACT GAA TTT CTT TTA AAT TTC AAC GAG TTT ATT GAT AGA AAA      2068
Asn Asn Thr Glu Phe Leu Leu Asn Phe Asn Glu Phe Ile Asp Arg Lys
        665                 670                 675

ACT CCA AAC AAC CCA TCA TGT AAC GCG GAT TTG ATT AAT AGA GTC TTG      2116
Thr Pro Asn Asn Pro Ser Cys Asn Ala Asp Leu Ile Asn Arg Val Leu
    680                 685                 690

CTT GAT GCA GGC TTT ACA AAT GAA CTT GTC CAA AAT TAC TGG AGT AAG      2164
Leu Asp Ala Gly Phe Thr Asn Glu Leu Val Gln Asn Tyr Trp Ser Lys
695                 700                 705                 710

CAG AAA AAT ATC AAG GGA GTG AAA GCA CGA TTT GTT GTG ACT GAT GGT      2212
Gln Lys Asn Ile Lys Gly Val Lys Ala Arg Phe Val Val Thr Asp Gly
                715                 720                 725

GGG ATT ACC AGA GTT TAT CCC AAA GAG GCT GGA GAA AAT TGG CAA GAA      2260
Gly Ile Thr Arg Val Tyr Pro Lys Glu Ala Gly Glu Asn Trp Gln Glu
            730                 735                 740

AAC CCA GAG ACA TAT GAG GAC AGC TTC TAT AAA AGG AGC CTA GAT AAT      2308
Asn Pro Glu Thr Tyr Glu Asp Ser Phe Tyr Lys Arg Ser Leu Asp Asn
        745                 750                 755

GAT AAC TAT GTT TTC ACT GCT CCC TAC TTT AAC AAA AGT GGA CCT GGT      2356
Asp Asn Tyr Val Phe Thr Ala Pro Tyr Phe Asn Lys Ser Gly Pro Gly
    760                 765                 770

GCC TAT GAA TCG GGC ATT ATG GTA AGC AAA GCT GTA GAA ATA TAT ATT      2404
Ala Tyr Glu Ser Gly Ile Met Val Ser Lys Ala Val Glu Ile Tyr Ile
775                 780                 785                 790
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CAA GGG AAA CTT CTT AAA CCT GCA GTT GTT GGA ATT AAA ATT GAT GTA          2452
Gln Gly Lys Leu Leu Lys Pro Ala Val Val Gly Ile Lys Ile Asp Val
                795                 800                 805

AAT TCC TGG ATA GAG AAT TTC ACC AAA ACC TCA ATC AGA GAT CCG TGT          2500
Asn Ser Trp Ile Glu Asn Phe Thr Lys Thr Ser Ile Arg Asp Pro Cys
            810                 815                 820

GCT GGT CCA GTT TGT GAC TGC AAA AGA AAC AGT GAC GTA ATG GAT TGT          2548
Ala Gly Pro Val Cys Asp Cys Lys Arg Asn Ser Asp Val Met Asp Cys
        825                 830                 835

GTG ATT CTG GAT GAT GGT GGG TTT CTT CTG ATG GCA AAT CAT GAT GAT          2596
Val Ile Leu Asp Asp Gly Gly Phe Leu Leu Met Ala Asn His Asp Asp
    840                 845                 850

TAT ACT AAT CAG ATT GGA AGA TTT TTT GGA GAG ATT GAT CCC AGC TTG          2644
Tyr Thr Asn Gln Ile Gly Arg Phe Phe Gly Glu Ile Asp Pro Ser Leu
855                 860                 865                 870

ATG AGA CAC CTG GTT AAT ATA TCA GTT TAT GCT TTT AAC AAA TCT TAT          2692
Met Arg His Leu Val Asn Ile Ser Val Tyr Ala Phe Asn Lys Ser Tyr
                875                 880                 885

GAT TAT CAG TCA GTA TGT GAG CCC GGT GCT GCA CCA AAA CAA GGA GCA          2740
Asp Tyr Gln Ser Val Cys Glu Pro Gly Ala Ala Pro Lys Gln Gly Ala
            890                 895                 900

GGA CAT CGC TCA GCA TAT GTG CCA TCA GTA GCA GAC ATA TTA CAA ATT          2788
Gly His Arg Ser Ala Tyr Val Pro Ser Val Ala Asp Ile Leu Gln Ile
        905                 910                 915

GGC TGG TGG GCC ACT GCT GCT GCC TGG TCT ATT CTA CAG CAG TTT CTC          2836
Gly Trp Trp Ala Thr Ala Ala Ala Trp Ser Ile Leu Gln Gln Phe Leu
    920                 925                 930

TTG AGT TTG ACC TTT CCA CGA CTC CTT GAG GCA GTT GAG ATG GAG GAT          2884
Leu Ser Leu Thr Phe Pro Arg Leu Leu Glu Ala Val Glu Met Glu Asp
935                 940                 945                 950

GAT GAC TTC ACG GCC TCC CTG TCC AAG CAG AGC TGC ATT ACT GAA CAA          2932
Asp Asp Phe Thr Ala Ser Leu Ser Lys Gln Ser Cys Ile Thr Glu Gln
                955                 960                 965

ACC CAG TAT TTC TTC GAT AAC GAC AGT AAA TCA TTC AGT GGT GTA TTA          2980
Thr Gln Tyr Phe Phe Asp Asn Asp Ser Lys Ser Phe Ser Gly Val Leu
            970                 975                 980

GAC TGT GGA AAC TGT TCC AGA ATC TTT CAT GGA GAA AAG CTT ATG AAC          3028
Asp Cys Gly Asn Cys Ser Arg Ile Phe His Gly Glu Lys Leu Met Asn
        985                 990                 995

ACC AAC TTA ATA TTC ATA ATG GTT GAG AGC AAA GGG ACA TGT CCA TGT          3076
Thr Asn Leu Ile Phe Ile Met Val Glu Ser Lys Gly Thr Cys Pro Cys
    1000                1005                1010

GAC ACA CGA CTG CTC ATA CAA GCG GAG CAG ACT TCT GAC GGT CCA AAT          3124
Asp Thr Arg Leu Leu Ile Gln Ala Glu Gln Thr Ser Asp Gly Pro Asn
1015                1020                1025                1030

CCT TGT GAC ATG GTT AAG CAA CCT AGA TAC CGA AAA GGG CCT GAT GTC          3172
Pro Cys Asp Met Val Lys Gln Pro Arg Tyr Arg Lys Gly Pro Asp Val
                1035                1040                1045

TGC TTT GAT AAC AAT GTC TTG GAG GAT TAT ACT GAC TGT GGT GGT GTT          3220
Cys Phe Asp Asn Asn Val Leu Glu Asp Tyr Thr Asp Cys Gly Gly Val
            1050                1055                1060
```

TCT GGA TTA AAT CCC TCC CTG TGG TAT ATC ATT GGA ATC CAG TTT CTA 3268
Ser Gly Leu Asn Pro Ser Leu Trp Tyr Ile Ile Gly Ile Gln Phe Leu
1065 1070 1075

CTA CTT TGG CTG GTA TCT GGC AGC ACA CAC CGG CTG TTA TGACCTTCTA 3317
Leu Leu Trp Leu Val Ser Gly Ser Thr His Arg Leu Leu
1080 1085 1090

AAAACCAAAT CTGCATAGTT AAACTCCAGA CCCTGCCAAA ACATGAGCCC TGCCCTCAAT 3377

TACAGTAACG TAGGGTCAGC TATAAAATCA GACAAACATT AGCTGGGCCT GTTCCATGGC 3437

ATAACACTAA GGCGCAGACT CCTAAGGCAC CCACTGGCTG CATGTCAGGG TGTCAGATCC 3497

TTAAACGTGT GTGAATGCTG CATCATCTAT GTGTAACATC AAAGCAAAAT CCTATACGTG 3557

TCCTCTATTG GAAAATTTGG GCGTTTGTTG TTGCATTGTT GGT 3600

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 323 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCCCCTGCCA GTGGCCAAAC AGAAGCAGAA GTCGGGTAAT GAAATGACTA ACTTAGCCTT 60

TGAACTAGAC CCCCTAGAGT TAGAGGAGGA AGAGGCTGAG CTTGGTGAGC AGAGTGGCTC 120

TGCCAAGACT AGTGTTAGCA GTGTCACCAC CCCGCCACCC CATGGCAAAC GCATCCCCTT 180

CTTTAAGAAG ACAGAGCATG TGCCCCCCTA TGACGTGGTG CCTTCCATGA GGCCCATCAT 240

CCTGGTGGGA CCGTCGCTCA AGGGCTACGA GGTTACAGAC ATGATGCAGA AAGCTTTATT 300

TGACTTCTTG AAGCATCGGT TTG 323

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CCTATTGGTG TAGGTATACC AACAATTAAT TTAAGAAAAA GGAGACCCAA TATCCAG 57

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 180 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..132

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
TGG TCC TTT GCC TGC GCC TGT GCC GCC TTC ATC CTC CTC TTT CTC GGC        48
Trp Ser Phe Ala Cys Ala Cys Ala Ala Phe Ile Leu Leu Phe Leu Gly
 1               5                  10                  15

GGT CTC GCC CTC CTG CTG TTC TCC CTG CCT CGA ATG CCC CGG AAC CCA        96
Gly Leu Ala Leu Leu Leu Phe Ser Leu Pro Arg Met Pro Arg Asn Pro
            20                  25                  30

TGG GAG TCC TGC ATG GAT GCT GAG CCC GAG CAC TAACCCTCCT GCGGCCCTAG      149
Trp Glu Ser Cys Met Asp Ala Glu Pro Glu His
        35                  40

CGACCCTCAG GCTTCTTCCC AGGAAGCGGG G                                     180
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid;
(A) DESCRIPTION: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
AATTCGGTAC GTACACTCGA GC                                               22
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid;
(A) DESCRIPTION: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
GCTCGAGTGT ACGTACCG                                                    18
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid;
(A) DESCRIPTION: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
CCATGGTACC TTCGTTGACG                                                  20
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid;
(A) DESCRIPTION: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AATTCGTCAA CGAAGGTACC ATGG 24

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 249 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CGGTACGTAC ACTCGAGCGA CTGTGTCATG ATGTTTATTA GTGATGACAG TGAGGTGAGG 60

CAGGGGCTTG TGGAGCATGC TCTGTAGGTC ACACACTAGA GCCATAAGGC AAGAGTAGGC 120

GGGGAGACAG GTCCTCTGTG CCCTGTCTCT CCCCATCTAA CCCTAACCTA ACAAGCGGTA 180

GTTATGAGTC AGGGAACAAC GTCTGGAGCC CCGTCCTCCA AAGATGTTTG AGGGACAAGA 240

ACAGAAATG 249

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 402 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CCACCTAGCA CGGGTTCGTT CCCCTCTCCC GGCCTGGCCC GGGCTCCCCG GTGGCCGCCG 60

CCCCCTCGCC GCCCATCTCT GGACTCAGAC GTCTCCCTGG AGGAGGACCG GGAGAGTGCC 120

CGGCGTGAAG TAGAGAGCCA GGCTCAGCAG CAGCTCGAAA GGGCCAAGCA CAAACCTGTG 180

GCATTTGCGG TGAGGACCAA TGTCAGCTAC TGTGGCGTAC TGGATGAGGA GTGCCCAGTC 240

CAGGGCTCTG GAGTCAACTT TGAGGCCAAA GATTTTCTGC ACATTAAAGA GAAGTACAGC 300

AATGACTGGT GGATCGGGCG GCTAGTGAAA GAGGGCGGGG ACATCGCCTT CATCCCCAGC 360

CCCCAGTGCC TGGTGAGCAT CCGCTCAAAC AGGAGCAGAA GG 402

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid;
(A) DESCRIPTION: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CTCAGTACCA TCTCTGATAC CAGCCCCA 28

WHAT IS CLAIMED IS:

1. An isolated DNA fragment, comprising a sequence of nucleotides that encodes an α_1-subunit of a human calcium channel.

2. The DNA fragment of claim 1, wherein the α_1-subunit is a human neural calcium channel α_1 subunit.

3. The DNA fragment of claim 1, wherein the α_1 subunit is an α_{1D} type α_1-subunit, α_{1C} type α_1-subunit, α_{1B} type α_1-subunit or an α_{1A} type α_1-subunit.

4. An isolated DNA fragment, comprising a sequence of nucleotides that encodes an α_2-subunit of a human calcium channel.

5. The DNA fragment of claim 4, wherein the human calcium channel is a human neural calcium channel, a human skeletal muscle calcium channel or a human aortic calcium channel.

6. The DNA fragment of claim 4, wherein the α_2 subunit is produced by alternative processing of a primary transcript that includes DNA encoding the amino acids set forth in sequence ID No. 11 and the DNA of Sequence ID No. 13 inserted between nucleotides 1624 and 1625 of Sequence ID No. 11.

7. The DNA fragment of claim 4, wherein the α_2 subunit is an α_{2a}, α_{2b}, α_{2c}, α_{2d}, or an α_{2e} subunit.

8. An isolated DNA fragment, comprising a sequence of nucleotides that encodes a β-subunit of a human calcium channel.

9. The DNA fragment of claim 8, wherein the subunit is a β_1 or β_3 subunit.

10. The DNA fragment of claim 8, wherein the β subunit is produced by alternative processing of a primary transcript that includes DNA encoding the amino acids set forth in sequence ID No. 9, but including the DNA set forth in Sequence ID No. 12 inserted in place of nucleotides 615-781 of Sequence ID No. 9.

11. The DNA fragment of claim 8, wherein the subunit is encoded by a transcript that lacks one or more sequences

of nucleotides selected from the group consisting of nucleotides 14-34 of Sequence ID No. 12, nucleotides 13-34 of Sequence ID No. 12, nucleotides 35-55 of Sequence ID No 12, nucleotides 56-190 of Sequence ID No. 12 and nucleotides 191-271 of Sequence ID No. 12.

12. An isolated DNA fragment, comprising a sequence of nucleotides that encodes a γ-subunit of a human calcium channel.

13. A eukaryotic cell, comprising heterologous DNA that encodes at least one subunit of a human calcium channel selected from the group consisting of an α_1-subunit, a β subunit, an α_2-subunit and a γ subunit.

14. The eukaryotic cell of claim 13, wherein the heterologous DNA encodes an α_1-subunit of a human calcium channel.

15. The eukaryotic cell of claim 13, wherein the α_1-subunit is an α_{1A} subunit, an α_{1B} subunit, an α_{1C} subunit or an α_{1D} subunit.

16. The eukaryotic cell of claim 13, wherein the heterologous DNA encodes an α_2-subunit of a human calcium channel.

17. The eukaryotic cell of claim 16, wherein the calcium channel is a human skeletal muscle calcium channel or a human aortic calcium channel.

18. The eukaryotic cell of claim 13, wherein the heterologous DNA encodes a β-subunit of a human calcium channel.

19. The eukaryotic cell of claim 13, wherein the heterologous DNA encodes a γ-subunit of a human calcium channel.

20. The eukaryotic cell of claim 13 which has a functional heterologous calcium channel that contains at least one subunit encoded by the heterologous DNA.

21. The eukaryotic cell of claim 20, wherein at least one subunit encoded by the heterologous DNA is an α_1-subunit of a human calcium channel.

22. The eukaryotic cell of claim 21, wherein at least two subunits are encoded by the heterologous DNA and the subunits encoded by the heterologous DNA, in addition to the α_1-subunit, are a β-subunit or an α_2-subunit.

23. The eukaryotic cell of claim 22, wherein the calcium channel contains at least three subunits that are encoded by the heterologous DNA.

24. The eukaryotic cell of claim 23, wherein the calcium channel contains at least four subunits encoded by the heterologous DNA.

25. The eukaryotic cell of claim 13 selected from the group consisting of HEK 293 cells, Chinese hamster ovary cells, African green monkey cells, and mouse L cells.

26. The eukaryotic cell of claim 20 selected from the group consisting of HEK 293 cells, Chinese hamster ovary cells, African green monkey cells, and mouse L cells.

27. A eukaryotic cell with a functional, heterologous calcium channel, produced by a process comprising introducing at least one RNA transcript selected from the group consisting of a first RNA which is translatable in said cell into an α_1-subunit of a human calcium channel, a second RNA which is translatable in said cell into a β-subunit of a human calcium channel, a third RNA which is translatable in said cell into an α_2-subunit of a human calcium channel, and a fourth RNA which is translatable in said cell into a γ-subunit of a human calcium channel.

28. The eukaryotic cell of claim 27 which is an amphibian oöcyte.

29. A method for identifying a compound that modulates the activity of a calcium channel, comprising;

suspending a eukaryotic cell which has a functional, heterologous calcium channel, in a solution containing said compound and a calcium channel selective ion:

depolarizing the cell membrane of said cell; and

detecting the current flowing into said cell,

wherein:

the heterologous calcium channel includes at least one human calcium channel subunit encoded by DNA or RNA that is heterologous to said cell,

the current that is detected is different from that produced by depolarizing the same or a substantially identical cell in the presence of the same calcium channel selective ion but in the absence of said compound.

30. The method of claim 29, wherein prior to the depolarization step the cell is maintained at a holding potential which substantially inactivates calcium channels which are endogenous to said cell.

31. The method of claim 30, wherein:

the cell is an amphibian oöcyte;

the heterologous subunits are encoded by RNA injected into the oöcyte; and

the heterologous subunits include an α_1-subunit and a β-subunit.

32. The method of claim 31, wherein the subunits encoded by said RNA further comprise an α_2-subunit, a γ-subunit or an α_2-subunit and a γ-subunit.

33. The method of claim 29, wherein the cell is an HEK cell and the heterologous subunit is encoded by heterologous DNA.

34. A substantially pure α_1-subunit of a human calcium channel encoded by the DNA of claim 1.

35. A substantially pure α_2-subunit of a human calcium channel encoded by the DNA of claim 4.

37. A substantially pure β-subunit of a human calcium channel encoded by the DNA of claim 8.

38. A substantially pure γ-subunit of a human calcium channel encoded by the DNA of claim 12.

39. The DNA fragment of claim 1, wherein the human calcium channel is a human neural calcium channel, a human skeletal muscle calcium channel or a human aortic calcium channel.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/06903

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 07 K 13/00, C 12 N 15/12, C 12 N 5/16, C 12 Q 1/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 07 K; C 12 N; C 12 Q

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	WO, A1, 9113077 (THE SALK INSTITUTE BIOTECHNOLOGY/INDUSTRIAL ASSOCIATES, INC.) 5 September 1991, see the whole document --	1-39
A	WO, A1, 8909834 (THE SALK INSTITUTE BIOTECHNOLOGY/INDUSTRIAL ASSOCIATES, INC.) 19 October 1989, see the whole document, cited in the application --	1-39
A	WO, A1, 8907608 (NEW YORK UNIVERSITY) 24 August 1989, see the whole document --	1-39

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24th November 1992	07 DEC 1992
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Mikael G:son Bergstrand

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,X	Proc. Natl. Acad. Sci. USA, vol. 89, January 1992, Susumu Seinoet al.: "Cloning of the alfa1 subunit of a voltage-dependent calcium channel expressed in pancreatic beta cells ", pp. 584-588, see especially the abstract, fig. 1	1-3,13-15,20-21,25-28
P,X	Dialog Information Services, file 154, Medline, 66-92/May, accession no. 07972010, Medline accession no. 92110010, Williams M.E. et al: "Structure and functional expression of alpha 1, alpha 2, and beta subunits of a novel human neuronal calcium channel subtype", & Neuron Jan 1992, 8 (1), p71-84	1-39
X	Dialog Information Services, file 154, Medline 66-91/May, accession no. 07846396, Medline accession no. 91365396, Powers P.A. et al: "Assignment of the human gene for the alpha 1 subunit of the cardiac DHP-sensitive Ca2+ channel (CCHL1A1) to chromosome 12p12-pter", & Genomics Jul 1991, 10 (3) p835-9	1-3,13-15
X	Proc. Natl. Acad. Sci. USA, vol. 86, May 1989, A. Lee Burns et al.: "Calcium channel activity of purified human synexin and structure of the human synexin gene ", pp. 3798-3802, see especially the abstract	1,4,8,12

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/US 92/06903

SA 63819

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 30/10/92
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A1- 9113077	05/09/91	NONE		
WO-A1- 8909834	19/10/89	AU-D-	3548089	03/11/89
		EP-A-	0424397	02/05/91
		JP-T-	4503152	11/06/92
WO-A1- 8907608	24/08/89	AU-D-	3193289	06/09/89
		EP-A-	0357730	14/03/90
		JP-T-	2503202	04/10/90
		US-A-	4950739	21/08/90